99B-117
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4.5 PSP Cover Sheet (Attach to the front of each proposal)

Proposal Title: Phylogeographic and Microsatellite Study of West Coast Estuarine Restricted Fish

Applicant Name: The Regents of the University of California

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Amount of funding requested: $ 385,808 for 3 years

Indicate the Topic for which you are applying (check only one box).

- ☐ Fish Passage/Fish Screens
- ☒ Habitat Restoration
- ☐ Local Watershed Stewardship
- ☐ Water Quality
- ☐ Introduced Species
- ☐ Fish Management/Hatchery
- ☐ Environmental Education

Does the proposal address a specified Focused Action? ____yes X no

What county or counties is the project located in? All coastal counties

Indicate the geographic area of your proposal (check only one box):

- ☐ Sacramento River Mainstem
- ☐ Sacramento Trib:
- ☐ San Joaquin River Mainstem
- ☐ San Joaquin Trib:
- ☐ Delta:
- ☐ East Side Trib:
- ☐ Suisun Marsh and Bay
- ☐ North Bay/South Bay:
- ☐ Landscape (entire Bay-Delta watershed)
- ☒ Other: All coastal California counties

Indicate the primary species which the proposal addresses (check all that apply):

- ☐ San Joaquin and East-side Delta tributaries fall-run chinook salmon
- ☐ Spring-run chinook salmon
- ☐ Fall-run chinook salmon
- ☐ Longfin smelt
- ☐ Steelhead trout
- ☐ Striped bass
- ☐ All chinook species
- ☐ All anadromous salmonids
- ☐ Winter-run chinook salmon
- ☐ Late-fall run chinook salmon
- ☐ Delta smelt
- ☐ Splittail
- ☐ Green sturgeon
- ☐ Migratory birds
- ☒ Other: Tidewater Goby, Bay Pipe Fish, Arrow Goby

Specify the ERP strategic objective and target (s) that the project addresses. Include page numbers from January 1999 version of ERP Volume I and II:

Species of native resident fish not assigned to priority group (page 184-346). Taxa considered are locally extinct or at risk but are not explicitly considered in your document.

Indicate the type of applicant (check only one box):

☐	State agency	☐	Federal agency
☐	Public/Non-profit joint venture	☐	Non-profit
☐	Local government/district	☐	Private party
☒	University	☐	Other:

Indicate the type of project (check only one box):

☐	Planning	☐	Implementation
☐	Monitoring	☐	Education
☒	Research		

By signing below, the applicant declares the following:

1.) The truthfulness of all representations in their proposal;

2.) The individual signing the form is entitled to submit the application on behalf of the applicant (if the applicant is an entity or organization); and

3.) The person submitting the application has read and understood the conflict of interest and confidentiality discussion in the PSP (Section 2.4) and waives any and all rights to privacy and confidentiality of the proposal on behalf of the applicant, to the extent as provided in the Section.

The Regents of the University of California
Printed name of applicant

Signature of applicant

Hardy Dhillon
Contract and Grant Officer

I -014122

I-014122

Title of Project : Phylogeographic and Microsatellite Study of West Coast Estuarine Restricted Fish

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Type of Organization:
University

Tax Status: Non-Profit

Tax Identification Number: 1956006143

Executive Summary

In this project we propose to examine fish taxa that were once common residents of the San Francisco Bay and adjacent habitats that are now either extirpated from the CALFED region entirely (tidewater goby, Swift et al.1989, 1993), or much reduced in the bay environment (bay pipefish, (Fritzsche 1980 and Swift et al. 1993). Thus the study will involve populations of fish that are primarily outside the CALFED study area. However, the project will have a number of direct and indirect benefits to CALFED. First, because impacts in the CALFED area have led to the reduction and extirpation of some of these fish, the population genetic issues pertaining to the fish that we propose to explore will be of importance if restocking is to be considered. Second, the issues of habitat preference and its relationship to population genetics of estuarine fishes may also be applicable to those fish species that are of special interest to the CALFED program. Perhaps of greatest interest in a conservation context, is that the extinction recolonization dynamics of tidewater gobies are now well documented. Thus through the work we propose, we will also be able to explore the population genetic consequences of variable regional extinction recolonization dynamics. This aspect of the research will have a strong impact on conservation of genetic variation in estuarine and stream fishes because it is one of few studies in which both the regional variation of and multiple instances of extinction/recolonization can be explored such that genetic consequences can be assayed. Lastly, this work will benefit a wide range of agencies by studying the genetic resources across a broad set of estuaries along the length of California and thus contributing to a more complete understanding of the entire system.

We propose a comparative phylogeographic and microsatellite study of four species of West Coast estuarine fish, whose ranges overlap, but that differ in habitat preference and fecundity. Using D-loop sequence, we will assess concordance of phylogeographic structure in *Eucyclogobius newberryi* (the tidewater goby), *Clevelandia ios* (the arrow goby), *Syngnathus leptorhynchus* (the bay pipefish) and *Leptocottus armatus* (the staghorn sculpin). In three of the taxa, the tidewater goby, arrow goby and bay pipefish, we will examine differences in population genetic structure resulting from habitat preference using microsatellites and D-loop sequence. In one taxon, the tidewater goby, we will use these classes of data to explore the population genetic consequences of different extinction/re-colonization dynamics across the range.

One objective of the phylogeographic study is to determine whether other taxa exhibit the deep break we find in our preliminary tidewater goby phylogeography. The tidewater goby, arrow goby and bay pipefish are estuarine restricted taxa in which one would anticipate stepping stone gene flow. The tidewater goby frequents small, often-closed, stream-mouth estuaries subject to hydrologic changes that result in frequent extinction/re-colonization of local populations. The sister taxon, arrow goby, inhabits more open, tidal, estuaries not subject to such dynamic changes in habitat and the bay pipefish inhabits eelgrass beds in open estuaries, a habitat subject to natural and anthropogenic disturbance. In contrast, the staghorn sculpin is a fecund and continuously distributed coastal fish that should lack the stepping-stone population qualities of the exclusively estuarine taxa in the analysis. Given fecundity and preferred habitat, we anticipate declining genetic subdivision in the following order: tidewater goby > bay pipefish>arrow goby> staghorn sculpin. With this hierarchical design we will be able to address questions about the commonality of pattern in phylogeography as well as the particular influence of reproductive mode and habitat preference on population structure and gene flow.

The tidewater goby is of special interest due to its federal endangered species status (currently under discussion for de-listing), and because there is documentation of extinction/re-colonization of populations. We will examine three hypotheses specific to the tidewater goby: 1) That genetic

diversity of local populations increases with latitude due to the influence of hydrology on metapopulation processes; 2) That recently re-colonized populations have lower genetic variation than those that have not gone extinct; and 3) That there is directionality of re-colonization associated with long-shore currents and this will be reflected in the genetic distances and measures of gene flow between "upstream" and "downstream" potential source populations.

For the D-loop phylogeographic project, samples will be collected throughout the range of the four species considered. For the more detailed assessment using microsatellites, sampling will be focused in a suite of estuaries around Point Reyes, Morro Bay and Point Conception/ Southern California Bight. To reduce D-loop sequencing, a restriction digest/SSCP screening protocol will be used. For population genetic analysis of D-loop and microsatellite data, exact tests will be conducted between all pairs of samples to assure appropriate grouping of samples. We will then examine structure within and between estuaries using a nested analysis of variance/F-statistic derived approach implemented by the program Arlequin. In addition, measures of genetic subdivision (e.g. F_{st}) will be calculated for all pairs of localities, and regressed against physical distances to assess isolation by distance.

This work addresses littoral dispersal processes, estuarine habitat partitioning and metapopulation dynamics. These issues are of fundamental interest relating to the evolutionary processes of marine taxa and of critical importance in fisheries management and the conservation of estuaries.

The total cost of the project is estimated to be $385,808.00

Our project not only considers the federally listed tidewater goby, but also addresses issues concerning other estuarine taxa, such as the bay pipefish. Thus it is of interest to a variety of federal regional and local agencies charged with the management of endangered species and genetic resources in the estuaries. On the federal and state levels, these include the EPA, the U.S. Fish and Wildlife Service, and the California Department of Fish and Game. Results of our work are of critical interest for management and policy decisions at local administrative units charged with the maintenance of genetic resources, including those at Vandenberg Air Force Base and the Camp Pendleton Marine Base. The latter location is of particular interest as our preliminary results (Fig.1) document that a genetically differentiated "stock" of tidewater goby now lives almost exclusively on Camp Pendleton. In another direction, pipefish and sea horses have been impacted by a range of human activities worldwide and are the subject of the international conservation initiative Project Seahorse based in England.

We have a well-equipped molecular lab suitable for addressing the range of molecular/population genetic questions proposed here. In addition we have substantial (deep -frozen) collections in hand including over 700 tidewater gobies (see Table 1), including substantial pre- and post-listing collections, as well as 163 arrow gobies from 6 locations, and over 500 individuals of bay pipefish and 95 staghorn sculpin from 13 locations. Substantial additional collecting will be essential for the project for which we already possess permits.

Project Description

Proposed Scope of the Work.

The estuaries on the West Coast form an array of separate habitats that are generally isolated from one another (Emmett et al., 1991; Swift et al., 1993). We propose to exploit the relative isolation of West Coast estuarine fish populations to explore gene flow along the coast. Specifically, we will examine the population genetics of four fish taxa using sequence from the D-loop (mitochondrial control region) and microsatellites. Using these data we will a) explore common geographic patterns that result from a shared history of dispersal, b) examine the effect of habitat preference on population structure and c) compare the impact of regionalized processes relating to extinction/re-colonization dynamics within a taxon.

Population structure along the West Coast has been examined in three broad categories based on the life history strategy of the organism. Those with a dispersal phase in their life history, those that lack a dispersal phase, and the partially or fully anadromous taxa such as many salmonids. Organisms with a dispersal phase in their life history generally exhibit little population subdivision or structure. This has been demonstrated for corals, barnacles and fish using allozymes (see Burton, 1998; Hellberg, 1995,1996), and in urchins using both allozymes and mitochondrial Cytochrome *c* Oxidase, subunit 1 (CO1) (Edmands et al., 1996). An extensive study of large bodied fish with early ontogenetic dispersal phases documents high gene flow values (Waples, 1987) that correlate positively with fecundity. This relationship that has now been confirmed in stream fish (Turner et al., 1996).

In contrast, organisms with a limited ability to disperse display considerably more population structure and phylogeographic patterning. The CO1 sequence of the upper intertidal copepod *Tigriopus californicus* from an array of coastal and channel islands sites exhibits dramatic sequence divergence and fixed allelic differences (Burton, 1997,1998; Burton and Feldman, 1981). This phylogeographic pattern reflects the long independent evolutionary histories of closely spaced populations.

Many taxa show dramatic population genetic differentiation between the Atlantic and Gulf coasts, a pattern that is concordant with faunal boundaries (Avise, 1992,1994). As such, it has been anticipated that breaks in gene flow on the West Coast would be closely associated with marine province boundaries, such as that found at Point Conception (Burton and Lee, 1994). Burton (1998) recently tabulated of the results of genetic studies over a range of invertebrate populations. This tabulation fails to identify any instances that document a break in the population genetic data that closely coincide with Point Conception.

The lack of a detectable break in these data may relate to complex physical and historical processes that also influence the phylogeography of marine organisms. Studies of current flow along the West Coast suggest that current structure is complex, multidirectional, and not very stable over time (Lynn and Simpson, 1987). The California Current is the major West Coast current flowing north to south. Both the Davidson Current and the California Undercurrent flow from south to north during much of the year. Several studies have documented the movement of drift markers from the Santa Monica Bay (Hickey, 1992) and the Santa Barbara channel (Henderschott and Winant, 1996). Surprisingly, these markers travel northwards around Point Conception, although the mass flux is thought to be southwards into the California Bight. As such, there appears to be opportunity for bi-directional, but unpredictable dispersal of marine organisms throughout the year. In addition to these major currents, long-shore currents associated with breaking waves may be particularly important for dispersal between small estuaries that are closely spaced. In contrast to the major currents, long-shore currents are predictable and are relatively uniform, in a southerly direction over stretches of the coastline.

Sea level may further complicate the historical phylogeographic pattern along the West Coast. For most of the last several hundred thousand years, sea level has been at values in the range of 50 to 100 meters lower than today (Shackleton, 1987). These low and fluctuating sea levels altered currents and coastal topography. Bathymetric analysis suggests there was some shallow water partitioning of the California Bight at these lower sea levels. Both allozyme work on the kelp fish *Gibbonsia* (Stepien and Rosenblatt, 1991), and a previously mentioned urchin study (Edmands et al., 1996) suggest there are coincident breaks in population genetic structure in this area.

The processes that influence population structure on the West Coast are clearly complex and the resolution of any genetic structure depends on the sensitivity of the techniques employed. A high-resolution analysis that simplifies aspects of the problem is required if a coherent signal regarding gene flow along the Pacific Coast is to be obtained. In this regard, we propose that studies of estuarine restricted taxa may have particular utility. The isolation of these discrete habitats should be ideal for maintaining geographic population structure. This spatial arrangement is likely to accord with the island and stepping stone type models of gene flow such that the analyses may be powerful and biologically relevant in this context. An emphasis on estuarine restricted, low fecundity "brooding" taxa should contribute to resolving population genetic structure and the analyses need only consider coastal habitats, as estuarine habitat is uncommon in the Channel Islands. Furthermore, a methodological approach that combines mitochondrial sequence and microsatellite data should allow for high resolution of the processes that influence population structure along the West Coast.

The four taxa selected for this study are the tidewater goby *Eucyclogobius newberryi* (Gobiidae); the arrow goby *Clevelandia ios* (Gobiidae), the bay pipefish *Syngnathus leptorhynchus* (Syngnathidae), and the staghorn sculpin *Leptocottus armatus* (Cottidae). These taxa are referred to by their common names throughout this proposal.

Tidewater gobies have a range from Smith River in northern California to Agua Hedionda in southern California (Swift et al., 1993, 1989; Lafferty et al., 1996). They most commonly inhabit estuaries, especially enclosed lagoons of coastal streams (Swift et al., 1993; Swenson and McCray, 1996). Spawning occurs predominantly in the summer months and the fish live for a year. Males defend eggs that are attached to the sides of burrows. The eggs hatch in about 9 days, releasing pelagic larvae that metamorphose and settle to the benthic substrate.

Arrow gobies have a range from Vancouver, British Columbia to Magdalena Bay, Baja California (Hart, 1973). The adults inhabit sheltered bays, estuaries, lagoons and tidal sloughs where they tolerate extremes of temperature and salinity. At low tide, the fish frequently exploit the burrows of the echiuran worm *Urechis*, and the ghost shrimps *Callianassa* and *Upogebia* (Hoffman, 1981). Males guard eggs attached to burrow walls, and the eggs hatch to produce pelagic larvae. The fish are not thought to migrate between locations (Prasad, 1958), however 10 day old pelagic larvae have been found in the California Current (Watson, 1996).

Bay pipefish have a range from southeastern Alaska to Baja California where they inhabit estuarine eelgrass beds (Orsi et al. 1991), a habitat that is very vulnerable to natural and anthropogenic disturbance. The female pipefish lays her eggs in the male's brood pouch where they are fertilized and reared. When fully developed, the free-swimming young are released from the brood pouch into the water column (Jones and Avise, 1997a; Fritzsche, 1980, 1984; Watson and Sandknop, 1996).

Staghorn sculpin have a range from the Gulf of Alaska to northern Baja, California (Jones, 1962; Hart, 1973; Armstrong et al., 1995) where they inhabit estuaries and the lower reaches of some coastal habitats (depending on seasonal fluctuations in salinity). The adults are restricted to marine water for breeding and produce thousands of eggs per spawn, which subsequently develop into pelagic larvae (Ambrose, 1996).

Laboratory Facilities.

The laboratory of David K. Jacobs is in the Department of Organismic Biology, Ecology and Evolution at UCLA. The total floor space of the lab is 1800 square feet with benches supplied with gas, vacuum, compressed air, distilled water and sinks and a utility area with 2 ventilation hoods, dishwasher and autoclave and drying ovens. And a 150 square foot cold room. The laboratory is equipped with (1) Forma Scientific -86°C ultra-cold chest freezer, multiple refrigerators, (1) -20°C freezer; a Millipore Water filtration unit, (1) Sorvall RC7-B refrigerated centrifuge with rotors, (1) Speedvac evaporator, (2) Eppendorf 5415C Microcentrifuges, (2) MJ Research Minicycler thermocyclers, (1) Perkin Elmer Gene amp 2400, (1) Pharmacia spectrophotometer, (1) Fisher BioTech FB650 power pack, (4) BioRad Sequi-Gen™ sequencing cells, (1) BioRad Model 583 gel dryer, (1) Hoeffer Scientific Instrument. SE1140 gel dryer, (2); BioRad PowerPac 300 power supplies, (2) BioRad mini-sub DNA cell rigs, (1) BioRad DNA sub cell rig, (1) Ultra Lum CCD camera and graphic printer, and (1) UV transilluminator. We also have the following computers: Apple Power Macintosh G3 (7300/200) personal computer with 64MB of physical memory, 1 GB of storage, and a dual-speed CD-ROM drive (1). Apple Power Macintosh 7100/66 personal computer with 16MB of physical memory, 260MB of storage, and a dual-speed CD-ROM drive and4 additional Macintosh computers.

Other Resources.

Departmental facilities include secretarial assistance, machine and electronics shops and an automated X-ray film processor. The automated sequencing will be conducted by The DNA Sequencing Facility, California State University at Northridge. This facility is competitively priced and we have an ongoing collaboration with this laboratory. The microsatellites will be run in the laboratory of Dr. Lanzaro at the University of Texas Medical Center, Galveston (see supplemental documentation). Dr. Lanzaro has an abundance of expertise in this area.

Location of the Project.

The estuaries treated in the analysis include sample localities in all the coastal counties of California. Two areas will be treated in greater depth in both D-loop work and microsatellite studies. These areas are 1) from just North of Pt. Conception in San Luis Obispo County south through San Diego County, and 2) a suite of estuaries around Pt. Reyes in Marin and Sonoma counties.

Ecological/Biological Benefits

Ecological/Biological Objectives.

Using sequences for the D-loop and microsatellites, we will examine the population genetics of four fish taxa that inhabit estuaries. These data will be analyzed to:

1. Explore common geographic patterns that result from a shared history of dispersal.
2. Examine the effects of habitat preference on population structure.
3. Analyze the impact of regionalized processes relating to extinction/re-colonization dynamics within a taxon.

An exploration of common geographic patterns that result from a shared history of dispersal (Objective 1) We predict that in marine organisms that have the ability to disperse, estuarine restricted organisms with low fecundity are the most likely to demonstrate geographic structure. To explore this prediction, we will reconstruct the intraspecific phylogenetic relationships within four fish species using mtDNA D-loop sequence. The resulting phylogeographic trees will be compared and analyzed for concordance of geographic structure. If these comparisons reveal concordance, the geographic

structure will be related to the physical processes that are considered to influence dispersal along the California Coast.

If no concordance of phylogeographic structure can be demonstrated, it would suggest that population genetic structure along the West Coast is not the consequence of a discrete set of correlated geographic dispersal processes. It may be that geographic differentiation only occurs in organisms with negligible dispersal ability, a perspective supported by a review of the marine population genetic data by Burton (1998). Such an outcome would be of considerable interest, as a wide range of basic scientific issues, as well as management decisions hinge on the nature and degree of common pattern of dispersal in nearshore marine taxa.

An examination of the effects of habitat preference on population structure (Objective 2)

To generate a more detailed understanding of the factors controlling gene flow and population subdivision, we will examine the impact of habitat preference and fecundity on population structure using mitochondrial D-loop sequence data and microsatellite loci. Based on our knowledge of the habitat preference of the selected taxa, we anticipate a declining trend in population subdivision in the following order: tidewater goby > bay pipefish >arrow goby> staghorn sculpin.

To address objective 2, we will employ microsatellites. Microsatellites are short stretches of DNA composed of di-, tri-, or tetranucleotide base pair repeat units arrayed in tandem (summary in Wright and Bentzen, 1994). Their high mutation rate and resultant high levels of length polymorphism make them particularly useful in discerning recent divergence and genetic diversity and in resolving details of geographic structure in populations (Walton et al., 1998; Van Oppen et al., 1997; Ruzzante et al. 1996). Microsatellites are being increasingly used in population level studies (review in Bruford et al., 1996; O'Connell and Wright, 1997; Park and Moran, 1994; Slatkin, 1995). These nuclear markers may be particularly informative in taxa that display male brooding and egg guarding as this kind of mating system (Jones and Avise, 1997a and b; Jones et al. 1998) may lead to greater female rather than male dispersal. In addition to providing a nuclear component to our research, the microsatellite study is designed to address specific comparative questions relating to habitat preference and population subdivision in the arrow goby, bay pipefish and tidewater goby.

The arrow goby and the bay pipefish inhabit the same estuaries and although both brood their young, they have very different habitat preferences. The arrow goby uses the burrows of other organisms for cover and achieves high population densities in the tidal flat habitat. These densities suggest that arrow gobies experience low barriers to dispersal within the estuary and possibly between closely spaced estuaries. In contrast, bay pipefish would appear to face greater challenges to dispersal. Bay pipefish depend heavily on eelgrass beds for camouflage and protection from predation, however, eelgrass beds are themselves extremely fragmented (Fritzche, 1980; B. Hoffman pers. comm.). We predict that the bay pipefish will exhibit reduced gene flow, greater population subdivision and greater isolation by distance than arrow gobies.

In addition to the bay pipefish comparisons, arrow gobies will also be compared with their putative sister taxon, the tidewater goby. Tidewater gobies preferentially inhabit small stream mouth estuaries. In the summer, the mouths of these estuaries are often closed off from the sea because of reduced stream flow and beach processes that generate berms. Most goby reproduction takes place during this period. As such, we predict that tidewater gobies will exhibit reduced gene flow, greater population subdivision and greater isolation by distance than arrow gobies.

A comparison of the impact of regionalized processes relating to extinction/re-colonization dynamics within a taxon (Objective 3)

Tidewater goby populations are highly vulnerable to changes in the hydrographic regime in the small streams they inhabit. Flooding and desiccation lead to extinction of whole populations. Because

of climate, the variance in stream flow is more extreme in Southern California as compared to Northern California. As such, the frequency of extinction/re-colonization events varies with both latitude and the range of stream size present in a region. The extinction/re-colonization events in tidewater gobies in Southern California have been documented (Fig 2; Lafferty et al., in press). Thus, we have a unique opportunity to examine the genetic structure of tidewater goby populations in the context of the history of extinction/re-colonization dynamics. This will be achieved using mitochondrial D-loop sequence and microsatellites.

The impact of extinction/re-colonization events on genetic diversity and population divergence will reflect both the number of individuals involved in re-colonization and any bias in terms of source. If large numbers of individuals are involved in re-colonization, gene flow could be enhanced and population differences diminished (Slatkin 1977, 1987). Alternatively, biases in recruitment resulting from re-colonization by adjacent populations (stepping stone) or directional recruitment could cause a reduction in the effective population size and increased genetic differentiation (Wade and McCauley, 1988; Whitlock and McCauley, 1990; Whitlock and Barton, 1997).

We propose to compare the genetic diversification within and between populations of tidewater gobies that inhabit sites with different histories of extinction/re-colonization. In addition, we will examine the impact of biases in re-colonization on the effective sizes of individual populations and neighborhoods. To this end, we will sample populations of tidewater gobies from three areas, Point Reyes, Point Conception and San Diego (Camp Pendleton).

The northern sites around Point Reyes have not been formally studied, but the more stable hydrographic regime makes it unlikely that the goby populations in this area are susceptible to extinction/re-colonization. Around Point Conception, several large systems harbor goby populations that are not subject to extinction. Between the larger systems lie smaller streams where recent extinction and re-colonization have occurred. In this region, we anticipate bias in the direction of re-colonization of extinct sites caused by long-shore processes. Such bias in combination with stepping stone behavior, and potentially small numbers of recruits should lead to founder effects and reduced genetic variation in, and increased differentiation between, the re-colonized populations (Dybdahl 1994). In contrast, the San Diego (Camp Pendleton) area consists of tightly spaced small stream-mouths, harboring goby populations that are subject to frequent extinction/ re-colonization events (Figure 2; Lafferty et al., in press). We suggest that tidewater gobies in this area approach a classic Levin's (1970) type metapopulation in terms of the comparable probabilities of extinction in all streams, and re-colonization from all local populations.

The tidewater goby data collected from the Point Reyes sites described in the previous section (Objective 2) will be used for these analyses. Tidewater gobies will be collected from 10 sites around Point Conception. Sites that harbor tidewater goby populations that have not gone extinct are the Santa Ynez River, Canada Santa Anita, Bell Canyon and Ventura River. The sites where recent extinction/recolonization has occurred include: Jalama Creek, Gaviota Creek, Refugio Canyon, Arroyo Burro, Ormond Beach, Malibu Creek. Microsatellites will be assayed in 50 individuals from each of these 8 sites. The San Diego (Camp Pendleton) sites are San Mateo Creek, San Onofre Canyon, Las Flores Lagoon, Hidden Lagoon, Aliso Canyon Lagoon, French Lagoon and the Santa Margarita River. The San Mateo Creek, San Onofre Canyon and Santa Margarita River sites are currently extinct so we will be limited to the fish collected in 1990 that are already in the laboratory. We anticipate studying a total of 300 fish from this group of 8 sites. As such, our sample sizes will be lower than the 50 fish per population optimum for microsatellites. Nonetheless, the preliminary D-loop data for these sites shows that these populations have low genetic variance such that large sample sizes may not be essential.

Significance of Proposed Research.

An exploration of common geographic patterns that result from a shared history of dispersal (Objective 1)

The comparative phylogeographic analysis of estuarine fish D-loop sequences will document evidence for the dispersal processes operating along the coast of California, a subject that is not well understood. A clarification of these processes is of critical scientific and applied interest. Dispersal is an essential component of marine evolution and ecology. Furthermore, an adequate understanding of dispersal processes are fundamental to fisheries management and the establishment of marine refuges and protected areas.

An examination of the effects of habitat preference on population structure (Objective 2)

Our preliminary data suggests that habitat preference strongly influence population genetic substructure. The proposed comparison of three taxa with different habitat preferences using detailed sampling regimes and state of the art microsatellite techniques, will resolve the effects of habitat substructure in the estuarine environment. The effects of habitat preference on genetic partitioning in the marine realm appears to be a relatively little explored issue of scientific interest, and to date no studies have directly addressed this issue along the West Coast. Estuaries are heavily impacted by human activities that have important implications for the federally endangered tidewater goby. Moreover, eelgrass beds, inhabited by the bay pipefish, support a unique fauna that is particularly sensitive to human activities. Thus, establishing a baseline of genetic partitioning within these estuarine habitats is of critical importance before they are degraded.

A comparison of the impact of regionalized processes relating to extinction/re-colonization dynamics within a taxon (Objective 3)

The known extinction/re-colonization dynamics of the tidewater goby in conjunction with the available samples presents a unique opportunity to study the impact of extinction/re-colonization dynamics on the population genetic structure of this taxon. For this purpose, the value of this system is greatly enhanced by the gradation in extinction/re-colonization dynamics with changing hydrography from the northern to the southern part of the range. Thus, over and above the status of this taxon as a federally endangered species, the elucidation of the relation between extinction/re-colonization dynamic and population genetic structure is exceptionally important in this area, as theory has far outpaced empirical tests. This work has obvious applied value in terms of the appropriate management of this taxon and in terms of the management of endangered taxa in the context of metapopulation process.

Benefits to Third Parties.

As mentioned in the executive summary, this study will be of direct benefit to those charged with the management of species and genetic resources involving the endangered tidewater goby. These include parties at the federal and state level as well as managers of such units as Vandenberg Air Force Base and the Camp Pendleton Marine Base. In addition there will be benefits to those with more general interest in the genetic subdivision of estuarine taxa and the marine processes that relate in such processes. Interested parties would include NOAA because of the estuarine topic, more specifically the National Marine Fisheries Service. More generally, the results of this works will interest parties concerned with mechanisms of population subdivision that involve the effects of different habitat type in aquatic systems and the population genetic consequences of extinction recolonization dynamics, such as those studying subjects that are encompassed by CALFED objectives. In addition as the

proposed questions are fundamental in the application of population biology to conservation, a very broad suite of organizations and individual researchers stand to benefit from the proposed study.

Technical Feasibility and Timing

This work is more comparative than other studies as it involves multiple techniques and several taxa. However, nothing about the techniques is particularly complex or novel and our preliminary data (Fig 1.) suggests that they will bear fruit that is of considerable importance. Thus, this comparative approach should provide clearer tests of hypotheses.

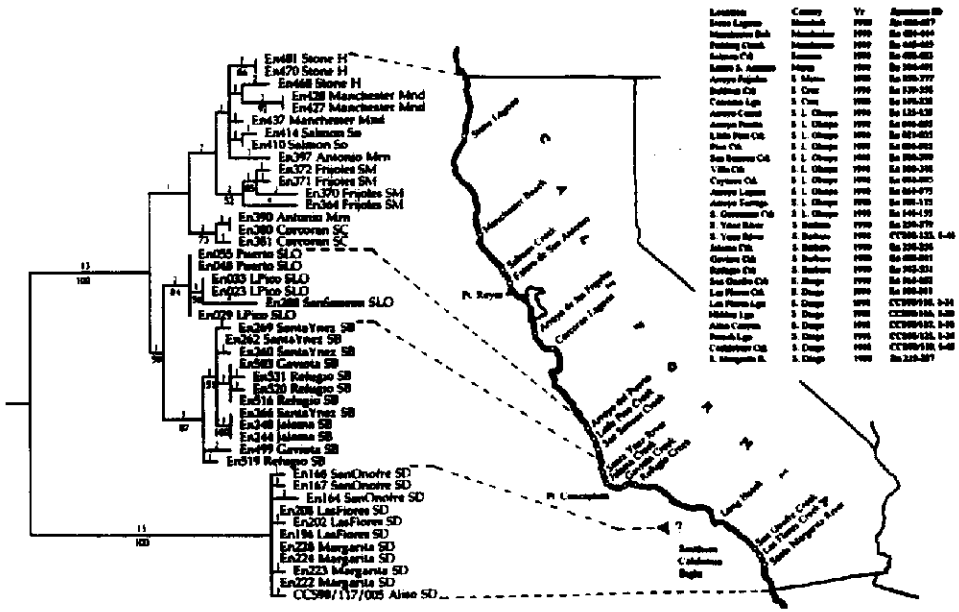

Figure 1. The phylogeography of tidewater goby based on 900bp of D-Loop sequence. Numbers above each branch indicate branch length, and those below indicate bootstrap support. Note the deep bifurcation between the Southern most San Diego clade and the rest of the tree which represents a 3% divergence. Also, note a monophyletic clade that wraps around Point Conception from Refugio to Santa Ynez, another related monophyletic clade in San Luis Obispo and a more northern clade extending from Santa Cruz to Humbolt counties. Sequence divergences are modest within the San Diego clade and there is greater haplotype distance and less geographic structure in the more northerly clades. Also, note that the major features of California Coast discussed in the text. These include Point Conception, the California Bight and Point Reyes. The tree was rooted with arrow goby *C. ios* (data not shown). Samples in hand are listed on the upper right hand corner of the figure. Specimen ID numbers reflect the numbers of specimens collected per site.

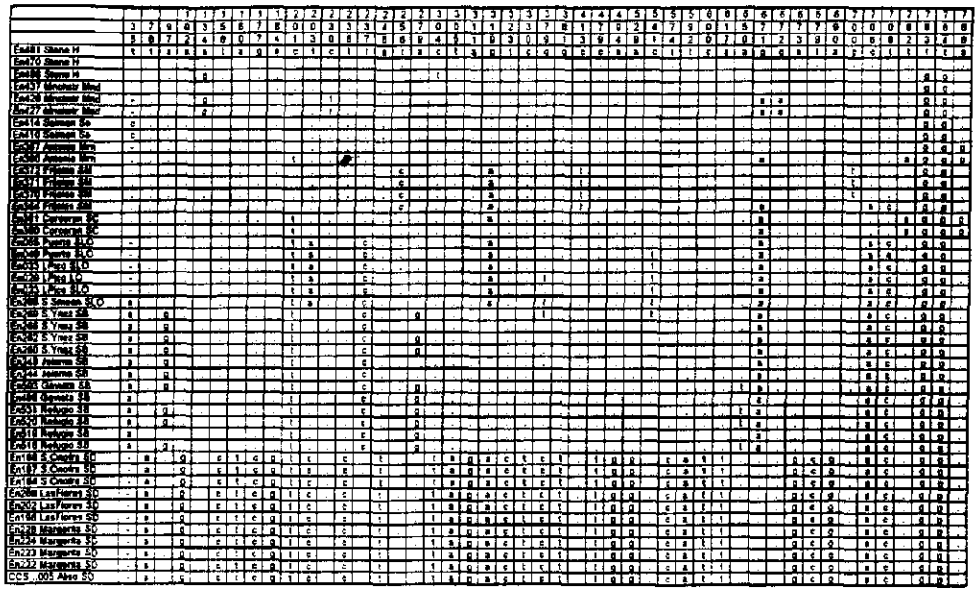

Table 1. List of phylogenetically informative positions in the tidewater goby D-loop data. Note that phylogenetic information is distributed throughout the sequence.

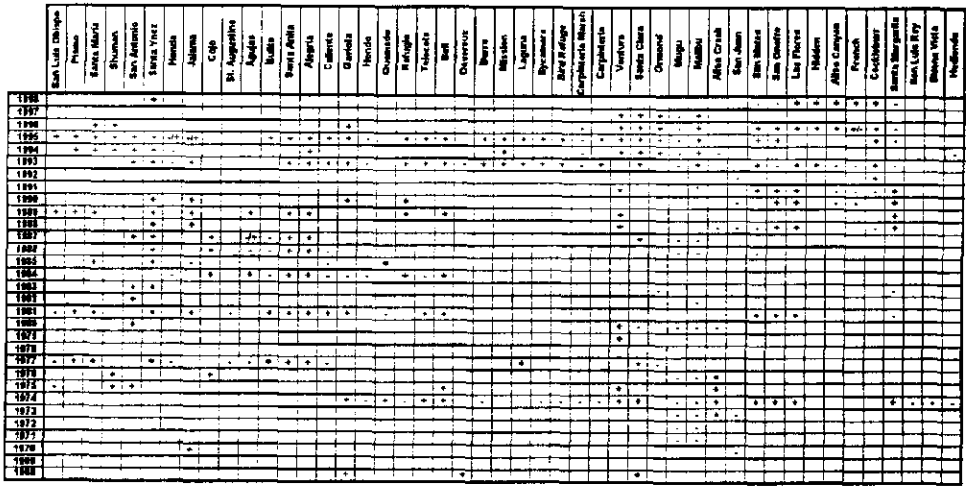

Table 2. List of tidewater goby sites in Southern California from Lafferty et al., in press, supplemented with our data. Plus symbols verifies the presence of fish based on a variety of data sources. Minus symbols show well supported absences of fish. A combination of plus and minus symbols suggests presence and absence of fish at different collection times within the same year.

Monitoring and Data Collection Methods

Biological/Ecological Objectives.

We propose to use two classes of analytical methods in this study, phylogeographic and population genetic. Although, both D-loop and microsatellite data can be applied to both types of study, mitochondrial sequence is of particular value in phylogeography due to its haploid non-recombining nature. As such, it should undergo a simple bifurcating evolutionary process, even within populations. Vertebrate mitochondrial DNA in general, and D-loop in particular, evolves relatively rapidly and therefore has the capacity to capture biogeographic structure within populations that have evolved over modest lengths of time (Lee et al., 1995; Avise, 1994). In contrast, microsatellites are repeat regions in nuclear DNA that evolve by slippage, with new alleles being generated very rapidly (as many 3 to 4 orders of magnitude faster than other loci). Due to their high degree of polymorphism and diploid nature, they are ideal for studies of parentage and fine-grained analysis of population genetic process. Unlike mitochondrial DNA, departures from Hardy-Weinberg can be assessed as a component of variance in microsatellite studies. However, because of the simple nature of microsatellite allelic differences (repeat number) and the rapidity of their evolution, microsatellite loci become saturated relatively quickly and do not retain a deep or ancient record of events. Thus, microsatellites and D-loop sequence compliment each other in that D-loop is optimal for reconstructing the phylogenetic relationship of closely related lineages and microsatellites permit the reconstruction of fine-scale population process. This combination of analyses is most comparable to the approach taken by Turner et al. (1996).

Monitoring Parameters, Data Collection, and Data Evaluation Approach.

We have a substantial fraction of the fish necessary to complete this study although a number of additional collections will be required. We are storing over 700 tidewater gobies from 31 locations (Figure 1); 163 arrow gobies from 6 locations; 500 bay pipefish from 13 locations and 95 staghorn sculpin from 13 locations from British Columbia to San Diego. We will compare the population genetics of arrow gobies and bay pipefish from an additional 12 sites in estuaries located around Point Reyes, Morro Bay and the Southern California Bight. Fifty individuals will be sampled from each of these 12 sites and assayed for 5 variable microsatellites.

DNA will be extracted from tissues following the CTAB and Proteinase K protocol in Dawson et al. (1998). The D-loop will be amplified using standard PCR conditions and primers from Lee et al. (1995) for all fish taxa except for the bay pipefish. Species specific primers have been designed in our lab to amplify the D-loop in the bay pipefish. Single Strand Conformation Polymorphism (SSCP) will be used to cost efficiently identify and screen for individuals with mutations, and hence nucleotide changes using a restriction digest, standard SSCP protocols, and SYBR Green stain to visualize the gel using UV light. Once an individual's genotype has been shown to have a novel genotype via this method, the PCR amplified DNA is isolated from the PCR mixture using QiaQuick PCR clean-up columns (Qiagen) and is eluted into 10mM Tris (pH 8.5) manual or automated sequencing. DNA sequences are manually generated using the Sequenase 2.0 DNA sequencing kit (Boehringer-Mannheim), and visualized with gel electrophoresis and autoradiography. The primers used to amplify the D-loop from fish DNA are used to directly sequence the PCR amplified D-loop DNA. In cases where the amplified product is in low concentration, the DNA is cloned prior to sequencing using the TOPO-TA cloning kit (Invitrogen). These templates are sequenced using universal primer sites located in the cloning vector flanking the inserted DNA. Primers to amplify microsatellites of tidewater gobies have been provided to us by Holly Mendonca. We anticipate these primers will also be useful in the sister taxa, the arrow goby. We have five microsatellite primer pairs for the bay pipefish, four of which

have been provided by Adam Jones (Jones and Avise, 1997a) and the last set from the stickleback *Gasterosteus aculeatus* has also shown to amplify in the bay pipefish (Taylor, 1998).

Data Evaluation.

Objective 1 will be explored by constructing phylogeographic trees using parsimony and maximum likelihood and PAUP version 4.0 software. Support for tree topologies will be assessed using bootstrap and Bremer support (Bremer, 1994). If phylogeographies are discordant between taxa, the statistical significance of this discordance will be assessed by comparing ratios of likelihood given the pairs of tree topologies and data sets (Felsenstien, 1981). A re-sampling approach will also be devised to assess how likely the observed geographic structure is, given the degree of genetic partitioning in the data.

We will use the exact test to generate and initiate comparison of all samples including those collected on different dates from the same locality. We will generate F_{st} statistics for all pairs of samples and these will be used in isolation by distance analyses (Hellberg, 1995, 1996; Turner et al., 1996). In the case of known instances of re-colonization "upstream and downstream" source sites will be compared to see if have they different properties in terms of regression of population differentiation versus distance. Population structures derived from both D-loop and microsatellites will be explored using the molecular analysis of variance. Here hierarchical structure within the data will be explored to examine neighborhoods (groups of samples) and test regional structure in the data.

The population genetic work to address objectives 2 and 3 will be conducted using the comprehensive population genetic software Arlequin version 1.1 (Schneider et al., 1997). This program implements a broad array of operations using both haplotype (mtDNA) and genetic (microsatellite) data. Specifically it incorporates the work of Goldstein et al. (1995) and Slatkin (1995), on the appropriate analytical approach for distance and F_{st} (R_{st}) analysis using microsatellite data. The program also permits facile analysis of hierarchical properties within genetic data, including Hardy Weinberg, sample and neighborhood components where appropriate.

Table 2. Monitoring and Data Collection Information

I) Biological/Ecological Objectives			
Hypothesis/Question to be Evaluated	Monitoring Parameter(s) and Data Collection Approach	Data Evaluation Approach	Comments/ Data Priority
Examine common history of dispersal process through phylogeography.	Complete collections & sequence D-loop of 4 estuarine fish	Phylogenetic analysis & tests of concordance	1
Examine the effects of habitat preference on population structure.	Compare microsatellite and D-loop data from arrow goby to bay pipefish and tidewater goby from suites of populations in the Southern California Bight and around Pt. Reyes.	Isolation by distance and hierarchical "F-statistic" analyses of population structure using the program Arlequin.	2
Analyze the population genetic impact of regionalized differences in extinction/re-colonization dynamics within a taxon.	Examine predictions for within population genetic diversity, and direction of recolonization generated from observed extinction recolonization dynamics (Lafferty et al.; Table 2).	Predictions of within population genetic variation will be tested using measures of effective population size and regression techniques to test for polarity of dispersal.	3

Local Involvement

In collecting tidewater gobies, we are very fortunate to be collaborating with Dr. Camm Swift, one of the foremost authorities on California stream and estuarine fish. The tidewater goby is a federally endangered species and all additional collections of this species will be coordinated by Dr. C. Swift who has a permit for this and related studies (supporting letter of collaboration, copies of portions of the federal and state permit and curriculum vitae attached). Additional collections of arrow gobies, bay pipefish and staghorn sculpin will be conducted under a state permit to Kristina Louie. All collections will be stored frozen and made available to other parties interested in these taxa in the future.

The primers to amplify microsatellites from tidewater gobies have been provided to us by Holly Mendonca who identified them as a component of her research for a Master's thesis at San Jose State (see attached letter and curriculum vitae).

There are many parties associated with and impacted by the outcome of this proposed project. Some of these impacted third parties are the following: the Federal Fish and Wildlife Service, the Department of the Interior, the National Oceanic and Atmospheric Administration's National Estuary Program, the California Department of Fish and Game, the Environmental Protection Agency, the National Biological Survey, many coastal California counties, and California State and National Parks.

Cost

Budget

Schedule

The proposed work will span over three years. Work during the first year, October 1999-September 30, 2000, will be devoted to completing the collections required for this study, although many of them are already in hand. In addition, during this period of time, we will complete collection of D-loop data for the four taxa, optimize the available microsatellite primers, as well as recover new primers as needed. During the second year, October 1, 2000 – September 30, 2001, we will complete our D-loop phylogeographic analyses and submit the study for publication and conduct initial microsatellite surveys across the targeted taxa. From October 1, 2001 to September 30, 2002, we will complete the remainder of the microsatellite surveys and their respective analyses required for hypotheses/tasks 2 and 3.

Cost-Sharing

We currently have no other support for the body of this work. We received $1,800 from the Genetics Resources Conservation Program (U.C. Davis) for the 1999-2000 year to support our collection of deep-frozen fish specimens and tissues.

Total Budget

Task	Direct Labor Hours	Direct Salary and Benefits	Service Contracts	Material and Acquisition Costs	Miscellaneous and other Direct Costs	Overhead and Indirect Costs	Total
Task I	1248	$ 19,531	0	$ 8,576	$ 1,364	$ 9,110	$ 38,581
Task II	1248	$ 19,531	0	$ 8,576	$ 1,364	$ 9,110	$ 38,581
Task III	3744	$ 51,533	0	$ 22,628	$ 3,600	$ 37,981	$ 115,742
Task IV	6240	$ 85,888	0	$ 37,713	$ 6,000	$ 63,303	$ 192,904
Project Management Task	0	0	0	0	0	0	
TOTAL	12,480	$176,483	$ -	$ 77,493	$ 12,328	$119,504	$ 385,808

Overhead and indirect costs have been calculated at the Off-campus rate of 26% MTDC. The rate is negotiated and approved by the Department of Health and Human Services, UCLA's cognizant federal agency. The modified total direct cost base consists of total direct costs less tuition and fee remission, equipment, capital expenditures, patient care, rental costs, scholarships, and fellowships and the portion of each subgrant and subcontract in excess of $25,000.
The indirect cost rate applies to both federal and state agencies.

COST SHARING:

UCLA will cost share 15% of Dr. David Jacobs' salary, benefits and indirect costs.

Table 4 - Quarterly Budget

	Oct-Dec 99	Jan-Mar 00	Apr-Jun 00	Jul-Sep 00	Oct-Dec 00	Jan-Mar 01	Apr-Jun 01	Jul-Sep 01	Oct-Dec 01	Jan-Mar 02	Apr-Jun 02	Jul-Sep 02	Totals
Task I	38,581												
Task II		12,860	12,860	12,860									38,581
Task III			14,467	14,467	21,702	21,702	21,702	21,702					38,581
Task IV					12,057	12,057	12,057	12,057	36,169	36,169	36,169	36,169	115,742
Project													192,904
Management													
Task													
Totals	38,581	12,860	27,327	27,327	33,759	33,759	33,759	33,759	36,169	36,169	36,169	36,169	385,808

Applicant Qualifications

The organization of the staff and other resources to be used in implementing this project.

David Jacobs is an assistant professor in the Department of Organismic Biology, Ecology and Evolution at the University of California Los Angeles. He received his Ph.D. at the Virginia Polytechnic Institute and State University in 1990. He has published in a wide range of areas involving the functional morphology of cephalopods the mechanisms and biological consequences of climate driven sea level fluctuation. He is currently developing molecular laboratory based research program focussing on comparative developmental genetics of invertebrates and on the population genetic structure of West coast marine invertebrates and Fishes.

Camm Swift is the foremost expert of California estuarine fishes and gobies. He has completed many of the primary works on the tidewater goby and is still involved in field and museum related research on the metapopulations of this endangered fish. He is an Emeritus associate curator of fishes at the Natural History Museum of Los Angeles County. Presently, he is a visiting assistant professor at Loyola Marymount University in Los Angeles and adjunct professor at the University of Southern California. Currently, he is also a consultant in conservation biology and fisheries of southern California freshwater and estuarine fishes. Dr. Swift also holds a California Department of Fish and Game contract to study the freshwater fishes of southern California. He has been under contract to study the status of the native freshwater fishes of southern California, including the status of the estuarine tidewater goby, a position that provides recommendations for preserves to maintain their existence.

Kristina Louie is a graduate student in the Department of Organismic Biology, Ecology and Evolution at the University of California Los Angeles with Dr. Jacobs. Her studies encompass conservation biology, ichthyology, estuarine habitats and evolutionary biology. She has collected the majority of bay pipefishes, arrow gobies and staghorn sculpins from British Columbia to San Diego for this project. She has generated some of the arrow goby data and all of the preliminary data for the bay pipefish and the staghorn sculpin and continues with these projects.

Michael Dawson is a graduate student in the Department of Organismic Biology, Ecology and Evolution at the University of California Los Angeles with Dr. Jacobs & Bill Hamner. His studies encompass evolutionary processes in estuaries and in the sea. He has conducted a number of studies on the marine lake of Palau as well as work on the population genetics of West coast marine taxa. His studies involve cnidarians and fish and he is largely responsible for the generation of the preliminary D-loop data set presented in Figure 1.

Collaborators

Microsatellite samples will be run at the University of Texas Medical Center, Galveston in the laboratory of Dr. Lanzaro, who has extensive experience with microsatellite studies. His laboratory is equipped with an ABI 377 and robotics dedicated to microsatellite work. We are extremely pleased that he has agreed to collaborate with us on this work and feel his facility will allow the rapid recovery of high quality data. Our samples will be run against size standards and scored using protocols already in place in Dr. Lanzaro's lab (see attached letter and Lanzaro et al., 1998).

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Appended General Laboratory methods

Extraction of DNA - Tissue is dissected from frozen or DMSO and sodium chloride preserved fish using a single edged sterile razor blade. DNA is extracted from the tissue by digestion in a mixture of CTAB and Proteinase K for approximately 4 hours at 55°C. Proteins are removed from the digest mixture by repeated extractions with phenol chloroform and the DNA is ethanol precipitated, dried and re-suspended in 10mM Tris (pH 8.5). This method has been used successfully to isolate DNA from a range of invertebrate and fish taxa (Dawson et al. 1998).

PCR amplification of D-loop DNA - For the tidewater goby, arrow goby and staghorn sculpin D-loop DNA is amplified from genomic DNAs using primers to the proline (CR-A) and phenolalanine (CR-M) tRNA's and standard PCR conditions (Lee et al., 1995). Attempts to use these primers on bay pipefish failed. However, D-loop DNA has been successfully amplified using primers to the tRNA threonine and conserved regions in the 12s gene.

Single Strand Conformation Polymorphism of D-loop DNA – Amplified D-loop DNAs are screened for unique genotype using a restriction digest and SSCP. Amplified D-loop DNAs are cut into small fragments (200-300 bp) using suitable restriction enzymes (Figure 2) and digesting at 37°C for 2 hours. To single strand the DNA, the digests are heated to 95°C for 3 minutes and immediately cooled on wet ice The samples are electrophoresed at 4°C in an 8% acrylamide/bis-acrylamide non denaturing gel. Migration patterns of the multiple digested strands are visualized using SYBR Green staining and the location of structural differences determined using a restriction map of the D-loop sequence.

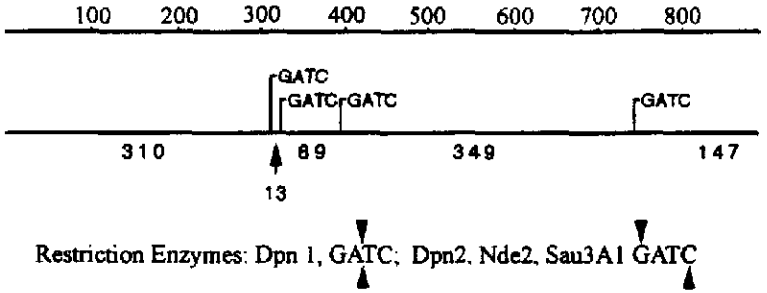

Figure 2. Restriction map of a tidewater goby D-loop indicating the restriction enzymes to be utilized for fragmenting D-loop sequences.

Preparation of D-loop DNA for Sequencing – Once the D-loop of an individual has been screened and shown to have a novel genotype, the PCR amplified DNA is isolated from the PCR mixture using QiaQuick PCR clean-up columns (Qiagen). The DNA is eluted into a small volume of 10mM Tris (pH 8.5) at high concentration and either manually sequenced or

sent to the DNA sequencing facility at California State University Northridge for automated sequencing.

Manual Sequencing of D-loop DNA - DNAs are manually sequenced using the Sequenase 2.0 DNA sequencing kit (Boehringer-Mannheim), and visualized with gel electrophoresis and autoradiography. The primers used to amplify the D-loop from fish DNA are used to directly sequence the PCR amplified D-loop DNA. The forward primer (CR-A) is used to sequence from the $tRNA^{pro}$ end of the control region to the poly-T region and the reverse primer (CR-M) used to sequence from the $tRNA^{phe}$ end of the control region. In cases where the amplified product is in low concentration, the DNA is cloned prior to sequencing using the TOPO-TA cloning kit (Invitrogen). These templates are sequenced using M13 Forward and M13 Reverse, universal primer sites located in cloning vector flanking the inserted DNA.

PCR amplification of microsatellites - The primers to amplify microsatellites from tidewater gobies have been provided to us by Holly Mendonca who identified them as a component of her research for a Master's thesis at San Jose State (see attached letter and curriculum vitae).

Locus	Repeating Unit	Product Size	No. of alleles
2	$(AC)_5AA(AC)_{10}$	152	2
8-1	$(ATCTCT)_5$	146	3
12	$(GT)_{12}$	133	3
14	$(GA)_7G(GA)_{11}$	233	3
22	Complex GT	281	Not analyzed

These primers are not highly variable, however to date they have been used to sample across only three closely spaced estuaries. We anticipate substantially more variation when they are applied to samples across the geographic range of tidewater gobies proposed in this study.

No specific primers are available to amplify microsatellites from arrow goby. However, our preliminary D-loop data support a close relationship between these putative sister taxa (C. Swift, pers. comm.). Arrow goby and tidewater goby sequences for the D-loop align smoothly with insertion of 2 single base gaps into the arrow goby sequence. The sequence exhibits 8% divergence from tidewater goby (compared to the 3% divergence within the tidewater goby), a reasonable divergence for closely related taxa. Thus, we will apply the primers developed for the tidewater goby to both the tidewater and arrow gobies. It should be noted that a number of studies of microsatellites in salmonids have successfully used microsatellite primers derived from other species and genera of salmonid fish (McConnell et al. 1995; Nielsen et al. 1997). In addition, a study of cross-species polymorphism reveals that microsatellite primer sites are maintained across widely divergent fish taxa and that locus heterozygosity is, in some cases, high across very divergent taxa (Rico et al., 1996). For example, the microsatellite *Gmo*02 derived from cod is highly polymorphic across 7 fish from 3 orders of teleosts as well as in sturgeon (Rico et al., 1996).

We have five microsatellite primer pairs for the bay pipefish, four of which have been provided by Adam Jones (Jones and Avise, 1997). These primers were previously used to assay parentage in the pipefish *S. scolvelli* where they were highly variable with 19 to 29

alleles per microsatellite. Although one of these primers sets is known to amplify in its congener, the bay pipefish (Jones and Avise, 1997a), the utility of the others has yet to be assessed. In addition, a microsatellite primer set from the stickleback *Gasterosteus aculeatus* has been shown to amplify in the bay pipefish (Taylor, 1998).

If the available primers prove inadequate for all or any of the three species in this study, we will have enriched microsatellite libraries constructed. An enriched library dramatically reduces the time required to screen for microsatellites (see attached letter from Ken Jones).

Analyzing PCR amplified microsatellites - Microsatellite samples will be run at the University of Texas Medical Center, Galveston in the laboratory of Dr. Lanzaro, who has extensive experience with microsatellite studies. His laboratory is equipped with an ABI 377 and robotics dedicated to microsatellite work. We are extremely pleased that he has agreed to collaborate with us on this work and feel his facility will allow the rapid recovery of high quality data. Our samples will be run against size standards and scored using protocols already in place in Dr. Lanzaro's lab (see attached letter and Lanzaro et al., 1998).

346 West Le Roy Avenue
Arcadia, CA 91007-6909
28 February 1999

Dr. David Jacobs
Department of Biology
University of California
Los Angeles, California 90024

Dear Dave:

With this letter I would like to confirm my commitment to collaborate with you in working on the variation and distribution of the tidewater goby and related estuarine gobies along the California coast. As we have discussed, you, your students, and I have several mutual interests in the systematics of the species of bay gobies. My interest in the conservation, morphological variation, and systematics of these species should complement your laboratories interest in evolutionary dynamics at the molecular level. There is, of course, the natural extension of working on the related "Chasmichthys" species in Japan, particularly in unraveling the cross Pacific relationships.

As you know I already have current federal and state permits to collect the species for all of our purposes in California, and permits from the state parks systems where they are appropriate. My current work on several of the populations in southern California has resulted in some collections for biochemical purposes and I am sure the others can be obtained easily in the near future, most likely by the end of the summer of 1999.

The biochemical data to be obtained will undoubtedly contribute largely to the conservation strategy for tidewater gobies and for restoration of estuaries in California. This will be in addition to the interesting metapopulational questions obviously presenting themselves with the semi-isolated populations of this species.

Please let me know if you need any more information.

With best regards,

Camm C. Swift
Emeritus Associate Curator,
Natural History Museum of Los Angeles County

I-014147

DEPARTMENT OF THE INTERIOR
U.S. FISH AND WILDLIFE SERVICE

FEDERAL FISH AND WILDLIFE PERMIT

2. AUTHORITY STATUTES
16 USC 1539(A)
16 USC 1533(D)

REGULATIONS (Attached)
50 CFR 17.22
50 CFR 17.32

1. PERMITTEE

CAMM C. SWIFT
348 W LEROY AVE
ARCADIA, CA 91007-6909

3. NUMBER: TE793644-3 — AMENDMENT

4. RENEWABLE: [X] YES [ ] NO

5. MAY COPY: [X] YES [ ] NO

6. EFFECTIVE: 09/29/1998

7. EXPIRES: 09/28/2002

8. NAME AND TITLE OF PRINCIPAL OFFICER (If not a business)

9. TYPE OF PERMIT: THREATENED AND ENDANGERED SPECIES

10. LOCATION WHERE AUTHORIZED ACTIVITY MAY BE CONDUCTED

ON LANDS SPECIFIED WITHIN THE BODY OF THE PERMIT.

11. CONDITIONS AND AUTHORIZATIONS.

A. GENERAL CONDITIONS SET OUT IN SUBPART D OF 50 CFR 13, AND SPECIFIC CONDITIONS CONTAINED IN FEDERAL REGULATIONS CITED IN BLOCK #2 ABOVE, ARE HEREBY MADE A PART OF THIS PERMIT. ALL ACTIVITIES AUTHORIZED HEREIN MUST BE CARRIED OUT IN ACCORD WITH AND FOR THE PURPOSES DESCRIBED IN THE APPLICATION SUBMITTED. CONTINUED VALIDITY, OR RENEWAL, OF THIS PERMIT IS SUBJECT TO COMPLETE AND TIMELY COMPLIANCE WITH ALL APPLICABLE CONDITIONS, INCLUDING THE FILING OF ALL REQUIRED INFORMATION AND REPORTS.

B. THE VALIDITY OF THIS PERMIT IS ALSO CONDITIONED UPON STRICT OBSERVANCE OF ALL APPLICABLE FOREIGN, STATE, LOCAL OR OTHER FEDERAL LAW.

C. VALID FOR USE BY PERMITTEE NAMED ABOVE.

D. Further conditions of authorization are contained in the attached Special Terms and Conditions.

[X] ADDITIONAL CONDITIONS AND AUTHORIZATIONS ALSO APPLY

12. REPORTING REQUIREMENTS

FIRST ANNUAL REPORT DUE: 01/31
See permit conditions for reporting requirements

ISSUED BY	TITLE	DATE
Larry Salata for	CHIEF - ENDANGERED SPECIES	09/29/1998

I -014148

I-014148

SPECIAL TERMS AND CONDITIONS FOR
Dr. Camm C. Swift

1. You were previously issued this permit on November 15, 1996. The terms and conditions set forth in that permit are hereby superseded by this amendment.

2. Acceptance of this permit serves as evidence that the permittee understands and agrees to abide by the "General Conditions for Native Endangered and Threatened Wildlife Species Permits," 50 CFR Part 13, 50 CFR 17.22 (endangered species) and/or 50 CFR 17.32 (threatened species), as applicable (copies attached). In addition, the permittee must have any other applicable State and Federal permits prior to the commencement of activities authorized by this permit.

3. Authorized to: take (capture and release, retain for lab studies, and sacrifice) the tidewater goby (*Eucyclogobius newberryi*); take (capture and release) the unarmored threespine stickleback (*Gasterosteus aculeatus williamsoni*); and take (harass by survey, capture and release) the California red-legged frog (*Rana aurora draytonii*) in conjunction with presence/absence surveys and scientific studies to enhance their survival as specified in the permittee's August 4, 1998, amendment request in accordance with the conditions stated below.

4. Permitted activities are restricted to lagoons, estuaries, and other areas of suitable habitat in coastal counties of California between and including Del Norte County and San Diego County, except where otherwise specified below.

Proposals to conduct activities pursuant to this permit at specific locations within the above referenced areas must be submitted in writing to the appropriate Fish and Wildlife Office (FWO) of the Fish and Wildlife Service at least 10 days prior to conducting such activities. The appropriate FWO is determined as follows:

> For Del Norte, Humboldt, and Mendocino Counties, contact the Coastal California Fish and Wildlife Office (CCFWO), 1125 16th Street, Suite 1209, Arcata, California 92008 (telephone: 707-822-7201; fax: 707-822-8411). For the Central Valley hydrographic basin and the coast ranges north of the Santa Cruz County line, contact the Sacramento Fish and Wildlife Office (SFWO), 3310 El Camino, Suite 130, Sacramento, California 95821 (telephone: 916-979-2725; fax: 916-979-2723). For areas from Santa Cruz County south to Los Angeles County north of the Angeles National Forest, contact the Ventura Fish and Wildlife Office (VFWO), 2493 Portola Road, Suite B, Ventura, California 93003 (telephone: 805-644-1766; fax: 805-644-3958). For areas from Los Angeles County including and south of the Angeles National Forest to San Diego County, contact the Carlsbad Fish and Wildlife Office (CFWO), 2730 Loker Avenue West, Carlsbad, California 92008 (telephone: 760-431-9440; fax: 760-431-9618).

I-014149

State of California
DEPARTMENT OF FISH AND GAME
1997 SCIENTIFIC COLLECTING PERMIT APPLICATION
License and Revenue Branch
3211 "S" Street
Sacramento, CA 95816
(916) 227-2225

DEPT OF FISH ... 1701 NIMBUS ... RANCHO CORDOVA CA ...

Logged 7/1/97

☒RENEWAL

PERMIT MUST BE IN IMMEDIATE POSSESSION WHILE COLLECTING

☐RESIDENT ☐NONRESIDENT ☐STUDENT ☐GOVERNMENT ☐COMMERCIAL FISHING CLASS STUDENT ☐COMMERCIAL FISHING CLASS FACULTY ☒OTHE

Read the instructions on the top copy before completing application. Type or print clearly

NAME: Camm Churchill Swift — DATE OF BIRTH: 09 / 29 / 1940

ADDRESS: 346 West Le Roy Avenue — CITY/STATE/ZIP: Arcadia, CA 91007-6909 — DAY TELEPHONE: (818) 447-5846

AGENCY/INSTITUTION/FIRM NAME: Department of Biology, Loyola Marymount University

ADDRESS: 7900 Loyola Blvd., — CITY/STATE/ZIP: Los Angeles, CA 90045-8220 — DAY TELEPHONE: (310) 338-5386

Students must obtain the signature of one sponsor; private collectors must have two signatures.

SPONSOR'S NAME AND TITLE | ORGANIZATION | DAY TELEPHONE ( )

ADDRESS | CITY/STATE/ZIP | SIGNATURE | DATE

SPONSOR'S NAME AND TITLE | ORGANIZATION | DAY TELEPHONE ( )

ADDRESS | CITY/STATE/ZIP | SIGNATURE | DATE

Do you have a current Federal permit to capture or band live birds or salvage dead birds in California? ☐Yes ☐No (If yes, attach a photocopy of permit)

If you are an authorized subpermittee, who is the master permit holder?

Describe the purpose, methods, number of specimens to be collected for your project and any other facts to justify the need to capture or possess any specimens. NOTE: Attach additional sheets if necessary.

Purpose: As a college biology teacher and adjunct Associate Curator of Fishes at the Natural History Museum of Los Angeles County, I am involved in surveying & conducting studies on a wide variety of fishes in the State. Many of these projects are in conjunction with CFG, U. S. Fish and Wildlife Service, and othe public and private agencies concerned with conservation and management of fishe in California. Attached are copies of my Federal Permit for the endangered tidewater goby, which includes incidental take of red-legged frogs and unarmore threespine stickleback, and my Memorandum of Understanding with CFG for take of Santa Ana Sucker, Arroyo chub, Santa Ana speckled dace,two forms of stickleback

Methods of Take: tidewater goby. ~~Thus I would like to be able to use all methods in~~

Where/How Specimens Kept: authorization 3 and to include marine and freshwater fishes anywhere in the state, excluding protected species not covered by my current permits

For each species you wish to take, please provide the information requested below, including exact location of take. Indicate "unknown" or "statewide" if appropriate. Provide additional sheets if necessary.

	COMMON NAME	SCIENTIFIC NAME	# Proposed to be removed from the wild	# AUTHORIZED (FOR DFG USE)	LOCATION AND COUNTY OF TAKE (Take is restricted to counties listed unless otherwise authorized.)
1	Potentially most species statewide, but mostly coastal lagoons throughout state and freshwater streams of southern California				
2					
3					
4					
5					
6					

I hereby declare that the information I have provided is true and correct to the best of my knowledge, and that I will comply with the provisions of Section 650, Title 14, California Code of Regulations pursuant to Section 1002, California Fish and Game Code. I understand this permit may be suspended or revoked by the Fish and Game Commission if I am convicted of, or plead guilty or nolo contendere to, a fish and game violation. I have read and agree to comply with the conditions and restrictions on the reverse side of this form.

Applicant's Signature: Camm Swift — Date: 30 May 1997

FOR DFG USE ONLY

AUTHORIZATIONS: 6, 6A #1

(See reverse for explanation)

RESIDENT SCIENTIFIC COLLECTING PERMIT 801104-02 Expires 12-31-97 Date Issued 7-10-97

IFD Approval: Arthur C. Baxter Jr. ☐ Letter permit attached Date 6/23/97 (SEE REVERSE)

WMD Approval: ☐ Letter permit attached Date

MRD Approval: Carrie E. Wills ☐ Letter permit attached Date 7/2/97

Routed to: Fish & Game 7/7 Date 6-30-97

Issued By: WM Date 7-10-97

White - Licensee | Pink - LRB | Yellow - Division | Green - Region | Blue - Suspense

I —014150

I-014150

CAMM CHURCHILL SWIFT, SS# 548-54-1835 CURRICULUM VITA Feb. 1999

346 West Leroy Avenue
Arcadia, CA 91007-6909
626 447-5846

Degrees: A. B. Zoology, U. C. Berkeley,
M. A., Zoology, U. Mich, Ann Arbor,
PhD, Fla. St. Univ, Tallahassee.

Academic Positions: (most recent first) Emeritus Assoc. Curator of Fishes, Nat. Hist. Mus. Los Angeles County, Visiting Asst. Prof., Biology, Loyola Marymount University, Los Angeles; Assoc. Curator Fishes, Natural History Museum of Los Angles County; simultaneously, Adj. Asst. Prof. Biology, Univ. S. California., and intermittently, Consultant in conservation biology and fisheries of southern California freshwater and estuarine fishes.

Research Interests: The biology, systematics, and paleontology of freshwater and estuarine fishes. Most recently the conservation biology of the federally endangered brackish water species, the tidewater goby, *Eucyclogobius newberryi*. This species is narrowly adapted to the upper brackish estuarine zone, and is scattered in semi-isolated populations over most of the coast of California.. Its biology and systematic relationships contribute to the understanding of the evolution of organisms into the estuarine and brackish water habitat, and the evolution of north Pacific coastal faunas in general. Habitat alteration in much of California has led to a federal endangered listing for this species. Its biology, projected backwards in time, has also contributed to the reconstruction of the original conditions of many California estuaries. This information is very important to the conservation and restoration the coastal marshes in California.

Grants: Research collaborator with Dr. George Dales. Use of fishes by the Harappan Culture Pakistan. Smithsonian Foreign Currency Program, 3 years; Co-principal investigator with L. Barnes, E. D. Mitchell, NSF EAR-7916508, Paleoecology of the Sharktooth Hill Bonebed, 2 years; Research collaborator with Drs. B. Berlin, J. L. Patton (co-principal investigators), NSF BSN-7916746, Field ethnobiological anthropology in the Peruvian Amazon, 3 years; Co-principal investigator with R. J. Lavenberg, NSF DEB-8008088, Development of Ichthyological Resources, 3 years; Co-principal investigator with J. Chovan, A national traveling exhibition on the biology of sharks, NSF Pre-College Education Grant MDR-8751868, 2 years.

Contracts: Freshwater fishes of southern California, survey and report. Calif. Dept. Fish and Game, 1 year, $75,000. Status of the native freshwater fishes of southern California, including the status of the estuarine tidewater goby, *Eucyclogobius newberryi*, with recommendations for preserves to maintain their existence. California Department of Fish and Game Contract FG-7455, one year. Cooperative Agreement, National Biological Service and Loyola Marymount University for study of the biology and habitat of tidewater goby on Vandenberg Air Force Base, Santa Barbara County. 1.5 years, $56,000. Prepare draft recovery plan tidewater goby, U. S. Fish and Wildlife Service, $5,600. Prepare historical analysis of coastal estuaries, habitat change, and restoration options, mouth Santa Maria River, Santa Barbara County, CA for California Department of Fish and Game Oil Response Team, $15,000. Co-author, U.S. Fish and Wildlife Service Recovery Plan for Endangered Unarmored Threespine Stickleback. Draft Survey

for the world conservation of fishes, for the International Union for Conservation of Nature and Natural Resources, Switzerland. Biology of the tidewater goby and management of aquatic exotic species, Marine Corps Base Camp Pendleton, CA. 3 years, $25,000.

Major Publications: 1986-present

1986. Swift, C.R. Gilbert, S.A. Bortone, G.A. Burgess, and R. W. Yerger. Zoogeography of the freshwater fishes of the southeastern United States: Savannah River to Lake Pontchartrain. pp. 213-265. In: C.H. Hocutt and E.O. Wiley, Eds., Zoogeography of North American Freshwater Fishes. Wiley Interscience, N.Y.

1989. Swift, J.S. Nelson, C. Maslow, and T. Stein. 1989. Biology and distribution of the tidewater goby, *Eucyclobius newberryi*, (Pisces, Gobiidae) of California. Nat. Hist. Mus. Los Angeles Co., Contrib. Sci. 404, 19 pp.

1993. Swift, T. H. Haglund, M. Ruiz, and R. Fisher. Status and distribution of the freshwater fishes of southern California. Bull. S. Calif. Acad. Sci., 92(3):101-168.

1996. Chapter 30. Distribution and migration. Pp. 595-630. In: Carl Bond. Biology of Fishes, (textbook) Second Edition. Harcort, Brace, and Co., Philadelphia.

1996. Lafferty, K., R. Swenson, and C. C. Swift. Tidewater goby; endangered species profile. Environmental Biology of Fishes, 46:254.

1999. K. Lafferty, C. C. Swift and R. Ambrose. Extinction and recolonization of populations of the endangered tidewater goby in southern California: a metapopulation? North American Journal of Fisheries Science (In press).

Manuscript in preparation: California Coastal Lagoons, historical and restoration perspectives. Contract for book with University of California Press.

Teaching experience: Comparative Vertebrate Anatomy, Ichthyology-Herpetology, Vertebrate Paleontology, Evolution, Embryology and development (molecular emphasis), Introductory Biology, Research Seminar in Estuarine Ecology, and non majors courses in Natural History of Southern California, Environmental Science, Introductory Biology, and Human Anatomy and Physiology, at Loyola Marymount University, Rio Hondo College, and Mount San Antonio College.

Professional Activities: Various positions, Amer. Soc. Ich. Herp., Cal-Neva Chapter of the Amer. Fish. Soc., S. Calif. Acad. Sci. Secretary, Vice-president, President and Fellow of the Academy. Host committees Los Angeles meetings Amer. Soc. Ich. Herp. (twice), Soc.Vert. Paleo., Cal-Neva Chapter AFS, and S. Calif. Acad. Sci. (three times). Currently Past President (until August, 1999) Cal-Neva Chapter AFS, Member Unarmored Threespine Stickleback Endangered Species Recovery Team (U.S. Fish and Wildlife Service). Research Associate, San Bernardino County Museums.

DEPARTMENT OF FISH AND GAME

1998 SCIENTIFIC COLLECTING PERMIT APPLICATION

License and Revenue Branch
1211 S Street,
Sacramento, CA 95816
(916) 227-2275

☒NEW

☐RENEWAL
(copy of previous permit and report of specimens collected must be attached)

PERMIT MUST BE IN IMMEDIATE POSSESSION WHILE COLLECTING

☐RESIDENT ☐NONRESIDENT ☒STUDENT ☐GOVERNMENT ☐COMMERCIAL FISHING CLASS STUDENT ☐COMMERCIAL FISHING CLASS FACULTY ☐OT

Read the instructions on the top copy before completing application. Type or print clearly.

NAME	DATE OF BIRTH (month/day/year)
KRISTINA D. LOUIE	11/13/74

ADDRESS	CITY/STATE/ZIP	DAY TELEPHONE
621 Circle Drive, South	Los Angeles CA 90095	(310) 206-7855

AGENCY/INSTITUTION/FIRM NAME

ADDRESS	CITY/STATE/ZIP	DAY TELEPHONE
		( )

College and commercial fishing class students must obtain the signature of one sponsor; private, scientific or educational collectors must have two signatures from professional staff of a college, museum or other scientific institution.

SPONSOR'S NAME AND TITLE	ORGANIZATION	DAY TELEPHONE
David K. Jacobs Professor	University of Calif., LA	(310) 206-7855

ADDRESS	CITY/STATE/ZIP	SIGNATURE	DATE
621 Circle Drive, South	Los Angeles, CA 90095		4.1

SPONSOR'S NAME AND TITLE	ORGANIZATION	DAY TELEPHONE

ADDRESS	CITY/STATE/ZIP	SIGNATURE	DATE
			( )

Do you have a current Federal permit to capture or band live birds or salvage dead birds in California? ☐Yes ☒No (If yes, attach a photocopy of permit)

Requested authorization(s) (see reverse side of form)

Describe the purpose, methods, number of specimens to be collected for your project and any other facts to justify the need to capture or possess any specimens. NOTE: Attach additional sheets if necessary. Attach a copy of your federal permit if you want to collect a federally listed threatened or endangered species.

Purpose: This proposed project seeks to investigate patterns & phylogenetic relationships and gene flow of estuarine fish taxa between estuaries along the western coast of N. America. Taxa from two different classes & estuarine systems will be compared - examining patterns of relationship using molecular sequence data. We will compare approximately 3 individuals of each taxa from 3 to 5 localities. Sample sizes of this nature are necessary to evaluate population genetic & historical dynamics. This data should reflect physical processes relating the community and longevity of the estuarine habitat itself. This type of assessment will be critical to understanding characteristics, management tactics of these threatened & endangered species.

Methods of Take: Minnow seine, dip net, plankton net

Where/How Specimens Kept: Frozen on dry ice, liquid nitrogen or preserved using alcohol & buffers

For each species you wish to take, please provide the information requested below, including exact location of take. Indicate "unknown" or "statewide", if appropriate. Provide additional sheets if necessary.

	COMMON NAME	SCIENTIFIC NAME	# Proposed to be removed from the wild	# AUTHORIZED (FOR DFG USE)	LOCATION AND COUNTY OF TAKE (take is restricted to coastal waters unless otherwise authorized)
1	Bay Pipefish	Syngnathus leptorhynchus	1500		all coastal counties
2	Tubesnout	Aulorhynchus flavidus	1500		all coastal counties
3	Long-jaw mudsucker	Gillichthys mirabilis	1500		all coastal counties
4	Arrow Goby	Clevelandia ios	1500		all coastal counties
5	Staghorn sculpin	Leptocottus armatus	1500		all coastal counties
6	(Please SEE Attached List)				

I hereby declare that the information I have provided is true and correct to the best of my knowledge, and that I will comply with the provisions of Section 650, Title 14, California Code of Regulations and Section 1002, California Fish and Game Code. I understand this permit may be suspended or revoked by the Fish and Game Commission if I am convicted of, or plead guilty or nolo contendere to, Fish and Game violations. I have read and agree to comply with the conditions and authorizations on the reverse side of this form.

Applicant's Signature _____ Date 4/14/98

FOR DFG USE ONLY

AUTHORIZATIONS: #6 may not take tidewater goby #3
(See reverse for explanation)

803048-01

BDD Approval _____ ☐Additional authorizations attached Date 5/6/98

IFD Approval Rebecca Miller ☐Additional authorizations attached Date 4/27/98

WMD Approval _____ ☐Additional authorizations attached Date _____

MRD Approval Carrie Nelson ☐Additional authorizations attached Date 5/20/98

Routed to Reg II - IFD MRD By: SH LRB Date 4/27/98

Issued By SH Date 6-4-98

White - Licensee Pink - LRB Yellow - Division Green - Region Blue - Suspense SH

I —0 1 4 1 5 3

I-014153

February 18,1999

Dave Jacobs
UCLA

Dear Dave:

This is to acknowledge our phone conversation of February 17, 1999 regarding our intention to collaborate on studies of the tidewater goby using the microsatellite loci I have identified and designed primers for in the tidewater goby genome. I have identified seven microsatellites and have successfully amplified tidewater goby DNA using five of the primer sets. Three have proven to be polymorphic in the populations I have examined, and the other two have not proven to be polymorphic. The other two primer sets have technical problems which could potentially be solved with some extra work, and they may also prove to be useful. The current study which identified and developed primers for these microsatellite loci have been used to examine the genetic makeup of a sampling of three different populations in Northern California, and was done to fulfill the Thesis requirement for the Masters Program at SJSU.

I am acknowledging my agreement to give these primer sets for these microsatellites which I have identified so they can be used in a grant submitted by Dave Jacobs to NSF, and that he may use them, if the grant is funded, for studies on DNA from fish he has collected at various locations over the range of the California coastline.

Sincerely,

Holly Mendonca

Holly Mendonca

Curriculum Vitae

Holly Lynn Mendonca
390 Madeline Court, Palo Alto, CA 94306
Tel. Work:650.859.2692 Home :650.424.1178

Education
M.S. Biology Projected graduation date: Spring 1999 San Jose State University
B.S. Marine Biology May 1989 Summa Cum Laude San Francisco State University

Selected Biology Departmental Honoree, May 1989
Member of Golden Key National Honor Society

Relevant Employment History

SRI International
Pharmaceutical Discovery Division, Cancer Biology Group
333 Ravenswood Avenue
Menlo Park, CA 94025
Supervisor: Dr. K. R. Laderoute
650.859.3080
Title: Biologist II
November 1989 - present

Areas of Expertise

* PCR ... sequencing of DNA
* Affinity and immunoprecipitation kinase assays
* Preparation of nuclear extracts, DNA and protein from cells; preparation of total RNA from cells, plasmid DNA from bacterial cultures
* S blots, N blots with radioactive labeling (32P) of nucleic acid or oligonucleotide probes; Western blots with chemiluminescent detection
* Transient and stable transfections of mammalian cells using liposome-based methods, calcium phosphate and electroporation
* Apoptosis assays using Hoescht stain, CHEF gels, and TUNEL assay
* Immunohistochemistry of cells and tissues...fluorescence microscopy
* Reporter gene activity assay...luciferase and CAT
* Electrophoresis: agarose gels to analyse DNA or RNA...1-d PAGE (mini and standard) to analyse protein...and others
* Tissue culture: maintenance and use of mammalian cell lines
* Computer skills...word processing for Mac and PC... spread sheets... Netscape...

Publications

K. R. Laderoute, H. L. Mendonca, J. M. Calaoagan, A. M. Knapp, A. J. Giaccia, and P. Stork. Mitogen-activated Protein Kinase Phosphatase-1 (MKP-1) Expression is Induced By Low Oxygen Conditions Found in Solid Tumor Microenvironments. A candidate MKP for the inactivation of hypoxia-inducible SAPK/JNK activity. J. Biol. Chem., 1998 (submitted).

K. R. Laderoute, J. M. Calaoagan, H. L. Mendonca, W. A. Ausserer, E. Y. Chen, A. J. Giaccia, and R. M. Sutherland. Early responses of SiHa human squamous carcinoma cells to hypoxic signals: Evidence of parallel activation of NF-kB and AP-1 transcriptional complexes. Int. J. Oncol. 8, 875-882, 1996.

V. K. Langmuir, K. Laderoute, H. L. Mendonca, R. M. Sutherland, T. K. Hei, S. X. Liu, E. J. Hall, M. A. Naylor, and G. E. Adams. Fused pyrazine mono-N-oxides as bioreductive drugs. II. Cytotoxicity in human cells and oncogenicity in a rodent transformation assay. Int. J. Radiat. Oncol. Biol. Phys. 34, 79-84, 1996.

N. S. Waleh, M. D. Brody, A. M. Knapp, H. L. Mendonca, E. M. Lord, C. J. Koch, K. R. Laderoute, and R. M. Sutherland. Mapping of the vascular endothelial growth factor-producing hypoxic cells in multicellular tumor spheroids using a hypoxic-specific marker. Cancer Res. 55, 6222,6226, 1995.

V. K. Langmuir and H. L. Mendonca. The combined use of 131I-labeled antibody and the hypoxic cytotoxin SR 4233 in vitro and in vivo. Radiat. Res. 132, 351-358, 1992.

V. K. Langmuir, B. W. Wessels, H. L. Mendonca, E. D. Yorke, and L. Montilla. Comparisons of micro-TLD dose measurements with predicted dose from 131I-labeled antibody. Med. Phys. 19, 1213-1218, 1992.

V. K. Langmuir and H. L. Mendonca. The role of radionuclide distribution in the efficacy of 131I-labeled antibody as modeled in multicell spheroids. Antibod. Immunoconjug. Radiopharm. 5, 273-283, 1992.

V. K. Langmuir, H. L. Mendonca, and D. V. Woo. Comparisons between two monoclonal antibodies that bind to the same antigen but have differing affinities: Uptake kinetics and 125I-antibody efficacy in multicell spheroids. Cancer Res. 52, 4728-4734, 1992.

V.K. Langmuir, H.L. Mendonca, J.-L. Vanderheyden, and F. M. Su. Comparisons of the efficacy of 186Re and 131I-labeled antibody in multicell spheroids. Int. J. Radiat. Oncol. Biol. Phys. 24, 127-132, 1992

Genetic Identification Services
9552 Topanga Canyon Blvd.
Chatsworth, CA 91311
(818) 718-9600 • (800) 362-4478 • Fax (818) 718-9620
gisemail@genetic-id-services.com
http://www.genetic-id-services.com

February 19, 1999

Dr. David Jacobs
Department of Biology
University of California
Los Angeles, CA 90095

Dear Dr. Jacobs:

This is to confirm GIS' willingness to assist you in producing microsatellite-enriched libraries to support your research in two species of gobies and one species of pipefish. Separate libraries would be produced that are enriched for at least four different motifs, including di-, tri-, and tetranucleotide microsatellites. As necessary, we can also assist in the identification of polymorphic loci through the design and testing of PCR primers developed from microsatellite-containing clones.

GIS has extensive experience in producing and using microsatellite-enriched libraries. At the present time, we have successfully produced libraries for nearly 60 species, including a wide range of both plant and animal taxa. The libraries consist of approximately 8,000 to 10,000 recombinant *E. coli* cells. Depending on the motif and species, enrichments often approach 100% for dinucleotides and 85% for tri- and tetranucleotide motifs. Thus, the libraries generally yield several thousand microsatellite-containing clones. Libraries are generally completed within four to six weeks of receipt of genomic DNA.

Please note that if we are unable to produce a library that is at least 50% enriched, there is no charge for our efforts. Please also note that we've never been unable to produce a highly enriched library in any of the species we've tried, so I'm confident that we will be successful in providing you the loci that you need.

I hope this answers any questions you might have. If you need any more information, please don't hesitate to contact me.

Sincerely yours,

Kenneth Jones
President

The University of Texas Medical Branch at Galveston

School of Medicine
Graduate School of Biomedical Sciences
School of Allied Health Sciences
School of Nursing

Marine Biomedical Institute
Institute for the Medical Humanities
UTMB Hospitals and Clinics

February 19, 1999

Dr. David Jacobs
Department of Biology
University of California
2203 Life Sciences
Los Angeles, CA 90095-1606

Dear Dave:

I have read your proposal on the population ecology and genetics of the Tidewater Goby and other estuarine fish and am very enthusiastic about collaborating with you on the use of microsatellites in defining the genetic structure of populations of these fish. We have been working on various projects dealing with insect population genetics and have been working with microsatellite DNA since 1993. My laboratory is well equipped for analyzing microsatellites with high throughput using automated DNA sequencing (ABI 377) and bio-robotics for handling large numbers of individual PCRs (Beckman Biomek 2000 robotic workstation). We have extensive experience working with software used for analyzing microsatellite gels (Genescan and Genotyper) and in formatting data sets for input into population genetics programs, such as Arlequin.

I would be pleased to host you or your co-workers in my laboratory for the purpose of conducting microsatellite analyses and in providing you with training on the aforementioned equipment and computer software. I enthusiastically agree to act as a collaborator with you. Best of luck on your proposal!

Sincerely,

Gregory C. Lanzaro
Associate Professor
Department of Pathology &
Center for Tropical Diseases

I-014158

BIOGRAPHICAL SKETCH

Gregory C. Lanzaro
Department of Pathology and Center for Infectious Diseases
University of Texas Medical Branch, Galveston, Texas 77555-0609

Education and training

Ph.D. 1986	Entomology	University of Florida
M.S. 1978	Entomology	University of Arizona
B.S. 1972	Biology	Kansas State University

Research and Teaching Experience:

7/98-12/90 : Post-Doctoral, Department of Entomology, University of California, Davis, C.A. Population genetics of snowpool *Aedes* mosquitoes.

1/91-6/93: MacArthur Fellow, Guest Researcher, National Institutes of Health, Laboratory of Malaria Research. Population genetics of *Anopheles gambiae* in West Africa.

7/93-8/95: Senior Staff Fellow, MacArthur Fellow, National Institutes of Health, Laboratory of Malaria Research. Molecular evolution of chromosome inversions in *Anopheles gambiae*; population genetics and molecular genetics of salivary peptides in the sand fly, *Lutzomyia longipalpis*.

8/95-8/97: Assistant Professor, Department of Pathology, University of Texas Medical Branch. Member, W.H.O. Collaborating Center for Tropical Diseases. Researched molecular population genetics of insect vectors of disease. Taught "Evolution of Infectious Disease" course.

9/97-present: Associate Professor, Department of Pathology, University of Texas Medical Branch, present Member Center for Infectious Diseases.

12/97: Appointed to Graduate Faculty, The University of Texas Graduate School of Biomedical Sciences at Galveston

Relevant Publications (11 of 29)

Mutebi, J-P, J.B. Alexander, I. Sherlock, J. Wellington, A.A. Souza, J. Shaw, E.F. Rangel and G.C. Lanzaro. Breeding structure of the sand fly *Lutzomyia longipalpis* (Neiva & Lutz) in Brazil. J. Amer. Soc. Trop. Med. Hyg. In Press.

Lanzaro, G.C., Ribeiro, J.M.C., Warburg, A., Shoemaker, C.B., Lopes, A.H.C.S., Soares, M. and R.G. Titus. Variation in the salivary peptide, maxadilan, from species in the *Lutzomyia longipalpis* complex. Insect Mol. Biol. In Press.

Lanzaro, G.C., Tourè, Y.T., Carnahan, J., Zheng, L., Dolo, G., TraorÈ, S., Petrarca, V., Vernick, K.D. and C.E. Taylor. Complexities in the genetic structure of *Anopheles gambiae* populations in west Africa as revealed by microsatellite DNA. Proc. Nat. Acad. Sci. USA. 95:14260-14265; 1998.

Yin, H., Mutebi, J. P., Marriott, S. and G.C. Lanzaro. Metaphase karyotypes and chromosome G-banding in members of the *Lutzomyia longipalpis* species complex. Med. Vet. Entomol. 13:1-6; 1998.

Mutebi, J.P., Rowton, E., Herrero, M.V., Ponce, C., Belli, A., Valle. S, and Lanzaro G.C. Genetic variability among Central American field populations of the sand fly, *Lutzomyia*

(*Lutzomyia*) *longipalpis* (Lutz & Meiva) (Diptera: Psychodidae). J. Med. Entomol. 35: 169-174, 1998.

Lanzaro G.C., Alexander, B., Mutebi, J.P., Montoya-Lerma, J. and Warburg, A. Genetic variation among natural and laboratory colony populations of *Lutzomyia longipalpis* from Columbia. Mem. Oswaldo Cruz. 93: 65-70, 1997.

Rongnoparut, P., Yaicharoen, S., Sirichotpakorn, N., Rattanarithikul, R., Lanzaro, G.C., and Linthicum, K. Microsatellite polymorphism in *Anopheles maculatus*, a malaria vector in Thailand. Am. J. Trop. Med. Hyg. 55: 589-594, 1996.

Lanzaro, G.C., Warburg, A. Genetic variability in phlebotomine sand flies: Possible implications for the epidemiology of leishmaniasis. Parasitol Today 11: 151-154, 1995.

Mathiopoulos, K.D., Lanzaro, G.C. Distribution of genetic diversity in relation to chromosomal inversions in the malaria mosquito, *Anopheles gambiae*. J Mol Evol 40: 578-584, 1995.

Lanzaro, G.C., Tourè, Y., Zheng, L., Kafatos, F., and Vernick, K. Microsatellite DNA and isozyme variability in a West African population of *Anopheles gambiae*. Insect Mol Biol 4: 105-112, 1995.

Warburg, A., Saravia, E., Lanzaro, G.C., Titus, R.G., and Neva, F. Saliva of *Lutzomyia longipalpis* sibling species differs in its composition and capacity to enhance leishmaniasis. Trans Roy Phil Soc 345:†261-267, 1994.

Most Recent Invited Presentations (Since 1995)

Keystone Symposia on Molecular & Cellular Biology, Taos, New Mexico, January 9-15, 1998. "Complexities in the genetic structure of vector populations: *Anopheles gambiae* in West Africa" In Symposium: "Toward the Genetic Manipulation of Insects".

Annual Meeting, Society for Vector Ecology. College Station, Texas, October 4-7, 1998. "Pitfalls and promise of microsatellite DNA in vector ecology and systematics" In Symposium: "Molecular Methods in Vector Systematics".

Annual Meeting, American Society of Tropical Medicine & Hygiene. Orlando, Florida, December†7-11, 1997. American Committee of Medical Entomology Symposium. "Identification of the real vector: sibling species complexes".

Second International Congress of Vector Ecology, Orlando, Florida, October 19-24, 1997. "The distribution of genetic variation in *Anopheles gambiae*: from genomes to populations".

Entomological Society of America, Annual Meeting, Louisville, Kentucky, December 8-12, 1996. "Highlights in Medical Entomology, 1995-96".

XX International Congress of Entomology, Florence, Italy, August 25-31, 1996. "Variation in the vasodilatory activity of saliva among populations of the sand fly, *Lutzomyia longipalpis.*"

MacArthur Foundation, Network on the Biology of Parasite Vectors, Institute, Fort Collin, Colorado, June, 1995. "Microsatellite DNA for the study of the population genetics of *Anopheles gambiae*".

U.S. Department of the Interior

Certifications Regarding Debarment, Suspension and Other Responsibility Matters, Drug-Free Workplace Requirements and Lobbying

Persons signing this form should refer to the regulations referenced below for complete instructions:

Certification Regarding Debarment, Suspension, and Other Responsibility Matters - Primary Covered Transactions - **The prospective primary participant further agrees by submitting this proposal that it will include the clause titled, "Certification Regarding Debarment, Suspension, Ineligibility and Voluntary Exclusion - Lower Tier Covered Transaction," provided by the department or agency entering into this covered transaction, without modification, in all lower tier covered transactions and in all solicitations for lower tier covered transactions.** See below for language to be used or use this form certification and sign. (See Appendix A of Subpart D of 43 CFR Part 12.)

Certification Regarding Debarment, Suspension, Ineligibility and Voluntary Exclusion - Lower Tier Covered Transactions - (See Appendix B of Subpart D of 43 CFR Part 12.)

Certification Regarding Drug-Free Workplace Requirements - Alternate I. (Grantees Other Than Individuals) and Alternate II. (Grantees Who are Individuals) - (See Appendix C of Subpart D of 43 CFR Part 12)

Signature on this form provides for compliance with certification requirements under 43 CFR Parts 12 and 18. The certifications shall be treated as a material representation of fact upon which reliance will be placed when the Department of the Interior determines to award the covered transaction, grant, cooperative agreement or loan.

PART A: Certification Regarding Debarment, Suspension, and Other Responsibility Matters-Primary Covered Transactions

CHECK____IF THIS CERTIFICATION IS FOR A PRIMARY COVERED TRANSACTION AND IS APPLICABLE.

(1) The prospective primary participant certifies to the best of its knowledge and belief, that it and its principals:

(a) Are not presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded by any Federal department or agency;

(b) Have not within a three-year period preceding this proposal been convicted of or had a civil judgment rendered against them for commission of fraud or a criminal offense in connection with obtaining, attempting to obtain, or performing a public (Federal, State or local) transaction or contract under a public transaction; violation of Federal or State antitrust statutes or commission of embezzlement, theft, forgery, bribery, falsification or destruction of records, making false statements, or receiving stolen property;

(c) Are not presently indicted for or otherwise criminally or civilly charged by a governmental entity (Federal, State or local) with commission of any of the offenses enumerated in paragraph (1)(b) of this certification; and

(d) Have not within a three-year period preceding this application/proposal had one or more public transactions (Federal, State or local) terminated for cause or default.

(2) Where the prospective primary participant is unable to certify to any of the statements in this certification, such prospective participant shall attach an explanation to this proposal.

PART B: Certification Regarding Debarment, Suspension, Ineligibility and Voluntary Exclusion - Lower Tier Covered Transactions

CHECK_X_ IF THIS CERTIFICATION IS FOR A LOWER TIER COVERED TRANSACTION AND IS APPLICABLE.

(1) The prospective lower tier participant certifies, by submission of this proposal, that neither it nor its principals is presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from participation in this transaction by any Federal department or agency.

(2) Where the prospective lower tier participant is unable to certify to any of the statements in this certification, such prospective participant shall attach an explanation to this proposal.

This form was electronically produced by Elite Federal Forms, Inc.

DI-2010
June 1995
(This form replaces DI-1953, DI-1954, DI-1955, DI-1956 and DI-1963)

PART C: Certification Regarding Drug-Free Workplace Requirements

CHECK X IF THIS CERTIFICATION IS FOR AN APPLICANT WHO IS NOT AN INDIVIDUAL.

Alternate I. (Grantees Other Than Individuals)

A. The grantee certifies that it will or continue to provide a drug-free workplace by:

(a) Publishing a statement notifying employees that the unlawful manufacture, distribution, dispensing, possession, or use of a controlled substance is prohibited in the grantee's workplace and specifying the actions that will be taken against employees for violation of such prohibition;

(b) Establishing an ongoing drug-free awareness program to inform employees about--
(1) The dangers of drug abuse in the workplace;
(2) The grantee's policy of maintaining a drug-free workplace;
(3) Any available drug counseling, rehabilitation, and employee assistance programs; and
(4) The penalties that may be imposed upon employees for drug abuse violations occurring in the workplace;

(c) Making it a requirement that each employee to be engaged in the performance of the grant be given a copy of the statement required by paragraph (a);

(d) Notifying the employee in the statement required by paragraph (a) that, as a condition of employment under the grant, the employee will --
(1) Abide by the terms of the statement; and
(2) Notify the employer in writing of his or her conviction for a violation of a criminal drug statute occurring in the workplace no later than five calendar days after such conviction;

(e) Notifying the agency in writing, within ten calendar days after receiving notice under subparagraph (d)(2) from an employee or otherwise receiving actual notice of such conviction. Employers of convicted employees must provide notice, including position title, to every grant officer on whose grant activity the convicted employee was working, unless the Federal agency has designated a central point for the receipt of such notices. Notice shall include the identification number(s) of each affected grant;

(f) Taking one of the following actions, within 30 calendar days of receiving notice under subparagraph (d)(2), with respect to any employee who is so convicted --
(1) Taking appropriate personnel action against such an employee, up to and including termination, consistent with the requirements of the Rehabilitation Act of 1973, as amended; or
(2) Requiring such employee to participate satisfactorily in a drug abuse assistance or rehabilitation program approved for such purposes by a Federal, State, or local health, law enforcement, or other appropriate agency;

(g) Making a good faith effort to continue to maintain a drug-free workplace through implementation of paragraphs (a) (b), (c), (d), (e) and (f).

B. The grantee may insert in the space provided below the site(s) for the performance of work done in connection with the specific grant:

Place of Performance (Street address, city, county, state, zip code)

621 Young Drive, South

Los Angeles, CA 90095

Check___if there are workplaces on files that are not identified here.

PART D: Certification Regarding Drug-Free Workplace Requirements

CHECK___IF THIS CERTIFICATION IS FOR AN APPLICANT WHO IS AN INDIVIDUAL.

Alternate II. (Grantees Who Are Individuals)

(a) The grantee certifies that, as a condition of the grant, he or she will not engage in the unlawful manufacture, distribution, dispensing, possession, or use of a controlled substance in conducting any activity with the grant;

(b) If convicted of a criminal drug offense resulting from a violation occurring during the conduct of any grant activity, he or she will report the conviction, in writing, within 10 calendar days of the conviction, to the grant officer or other designee, unless the Federal agency designates a central point for the receipt of such notices. When notice is made to such a central point, it shall include the identification number(s) of each affected grant.

DI-2010
June 1995
(This form replaces DI-1953, DI-1954 DI-1955, DI-1956 and DI-1983)

PART E: Certification Regarding Lobbying
Certification for Contracts, Grants, Loans, and Cooperative Agreements

CHECK_X_ IF CERTIFICATION IS FOR THE AWARD OF ANY OF THE FOLLOWING AND THE AMOUNT EXCEEDS $100,000: A FEDERAL GRANT OR COOPERATIVE AGREEMENT; SUBCONTRACT, OR SUBGRANT UNDER THE GRANT OR COOPERATIVE AGREEMENT.

CHECK___ IF CERTIFICATION FOR THE AWARD OF A FEDERAL LOAN EXCEEDING THE AMOUNT OF $150,000, OR A SUBGRANT OR SUBCONTRACT EXCEEDING $100,000, UNDER THE LOAN.

The undersigned certifies, to the best of his or her knowledge and belief, that:

(1) No Federal appropriated funds have been paid or will be paid, by or on behalf of the undersigned, to any person for influencing or attempting to influence an officer or employee of an agency, a Member of Congress, and officer or employee of Congress, or an employee of a Member of Congress in connection with the awarding of any Federal contract, the making of any Federal grant, the making of any Federal loan, the entering into of any cooperative agreement, and the extension, continuation, renewal, amendment, or modification of any Federal contract, grant, loan, or cooperative agreement.

(2) If any funds other than Federal appropriated funds have been paid or will be paid to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with this Federal contract, grant, loan, or cooperative agreement, the undersigned shall complete and submit Standard Form-LLL, "Disclosure Form to Report Lobbying," in accordance with its instructions.

(3) The undersigned shall require that the language of this certification be included in the award documents for all subawards at all tiers (including subcontracts, subgrants, and contracts under grants, loans, and cooperative agreements) and that all subrecipients shall certify accordingly.

This certification is a material representation of fact upon which reliance was placed when this transaction was made or entered into. Submission of this certification is a prerequisite for making or entering into this transaction imposed by Section 1352, title 31, U.S. Code. Any person who fails to file the required certification shall be subject to a civil penalty of not less than $10,000 and not more than $100,000 for each such failure.

As the authorized certifying official, I hereby certify that the above specified certifications are true.

SIGNATURE OF AUTHORIZED CERTIFYING OFFICIAL

Hardy Dhillon, Contract and Grant Officer
TYPED NAME AND TITLE

4/15/99
DATE

DI-2010
June 1995
(This form replaces DI-1953, DI-1954, DI-1955, DI-1956 and DI-1963)

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I-014163

[illegible] DEPARTMENT OF WATER RESOURCES [illegible] Agreement

Agreement No. _____

Exhibit _____

STANDARD CLAUSES -
INTERAGENCY AGREEMENTS

Audit Clause. For contracts in excess of $10,000, the contracting parties shall be subject to the examination and audit of the State Auditor for a period of three years after final payment under the contract (Government Code Section 8546.7).

Availability of Funds. Work to be performed under this contract is subject to availability of funds through the State's normal budget process.

Interagency Payment Clause. For services provided under this agreement, charges will be computed in accordance with State Administrative Manual Section 8752 and 8752.1.

Termination Clause. Either State agency may terminate this contract upon thirty days advance written notice to the other agency providing the agency has not been furnished for the reasonable expenses incurred up to the date of termination.

Severability. If any provision of this Agreement is held invalid or unenforceable by any court of final jurisdiction, it is the intent of the parties that all other provisions of this Agreement be construed to remain fully valid, enforceable, and binding on the parties.

Y2K Language. The Contractor warrants and represents that the goods or services sold, leased, or licensed to the State of California, its agencies, or its political subdivisions, pursuant to this Agreement are "Year 2000 compliant." For purposes of this Agreement, a good or service is year 2000 compliant if it will continue to fully function before, at, and after the Year 2000 without interruption and, if applicable, with full ability to accurately and unambiguously process, display, compare, calculate, manipulate, and otherwise utilize date information. This warranty and representation supersedes all warranty disclaimers and limitations and all limitations on liability provided by or through the Contractor.

DWR 4115 (REV. 1/99)

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OMB Approval No. 0348-0043

APPLICATION FOR FEDERAL ASSISTANCE

2. DATE SUBMITTED	Applicant Identifier
3. DATE RECEIVED BY STATE	State Applicant Identifier
4. DATE RECEIVED BY FEDERAL AGENCY	Federal Identifier

1. TYPE OF SUBMISSION

Application	*Preapplication*
Construction	Construction
[X] Non-Construction	[ ] Non-Construction

5. APPLICANT INFORMATION

Legal Name: The Regents of the University of California

Organizational Unit: Organismic Bio., Ecology & Evolution

Address *(give city, county, state, and zip code)*:
Box 951406
1400 Ueberroth
Los Angeles, Ca. 90095-1406

Name and telephone and E-mail number of the person to be contacted on matters involving this application *(give area code)*
Hardy Dhillon, (310) 825-0965; hdhillon@smet.ucla.edu

6. EMPLOYER IDENTIFICATION NUMBER (EIN):
9 5 - 6 0 0 6 1 4 3

7. TYPE OF APPLICANT: *(enter appropriate letter in box)* I

A. State
B. County
C. Municipal
D. Township
E. Interstate
F. Intermunicipal
G. Special District
H. Independent School Dist.
I. State Controlled Institution of Higher Learning
J. Private University
K. Indian Tribe
L. Individual
M. Profit Organization
N. Other (Specify)

8. TYPE OF APPLICATION:
[X] New [ ] Continuation [ ] Revision

If Revision, enter appropriate letter(s) in box(es): [ ] [ ]
A. Increase Award B. Decrease Award C. Increase Duration
D. Decrease Duration Other *(specify)*:

9. NAME OF FEDERAL AGENCY:
U.S. Department of the Interior

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER: [ ][ ].[ ][ ][ ]
TITLE: CALFED Bay-Delta program

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:
A Phylogeographic and Microsattelite Study of Pacific Coast Estuarian Restricted Fish

12. AREAS AFFECTED BY PROJECT *(cities, counties, states, etc.)*:
All coastal California Counties

13. PROPOSED PROJECT:

Start Date	Ending Date
10/01/99	09/30/02

14. CONGRESSIONAL DISTRICTS OF:

a. Applicant	b. Project
29th	Various

15. ESTIMATED TOTAL PROJECT FUNDING:

a. Federal	$	106,098 .00
b. Applicant	$	19,872 .00
c. State	$	.00
d. Local	$	.00
e. Other	$	.00
f. Program Income	$	.00
g. TOTAL	$	125,970 .00

16. IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?
a. YES. THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE _____
b. NO. [X] PROGRAM IS NOT COVERED BY E.O. 12372
[ ] OR PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. IS THE APPLICANT DELINQUENT ON ANY FEDERAL DEBT?
[ ] Yes If "Yes," attach an explanation. [X] No

18. TO THE BEST OF MY KNOWLEDGE AND BELIEF, ALL DATA IN THIS APPLICATION/PREAPPLICATION ARE TRUE AND CORRECT. THE DOCUMENT HAS BEEN DULY AUTHORIZED BY THE GOVERNING BODY OF THE APPLICANT AND THE APPLICANT WILL COMPLY WITH THE ATTACHED ASSURANCES IF THE ASSISTANCE IS AWARDED.

a. Typed Name of Authorized Representative	b. Title	c. Telephone number
Hardy Dhillon	Contract and Grant Officer	(310) 825-0695

d. Signature of Authorized Representative

e. Date Signed

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Standard Form 424 (Rev. 7-97)
Prescribed by OMB Circular A-102

I -014165

I-014165

ASSURANCES—NON-CONSTRUCTION PROGRAMS

Note: Certain of these assurances may not be applicable to your project or program. If you have questions please contact the awarding agency. Further, certain Federal awarding agencies may require applicants to certify to additional assurances. If such is the case, you will be notified.

As the duly authorized representative of the applicant I certify that the applicant:

1. Has the legal authority to apply for Federal assistance, and the institutional, managerial and financial capability (including funds sufficient to pay the non-Federal share of project costs) to ensure proper planning, management and completion of the project described in this application.

2. Will give the awarding agency, the Comptroller General of the United States, and if appropriate, the State, through any authorized representative, access to and the right to examine all records, books, papers, or documents related to the award; and will establish a proper accounting system in accordance with generally accepted accounting standards or agency directives.

3. Will establish safeguards to prohibit employees from using their positions for a purpose that constitutes or presents the appearance of personal or organizational conflict of interest, or personal gain.

4. Will initiate and complete the work within the applicable time frame after receipt of approval of the awarding agency.

5. Will comply with the Intergovernmental Personnel Act of 1970 (42 U.S.C. §§ 4728-4763) relating to prescribed standards for merit systems for programs funded under one of the nineteen statutes or regulations specified in Appendix A of OPM's Standards for a Merit System of Personnel Administration (5 C.F.R. 900, Subpart F).

6. Will comply with all Federal statutes relating to nondiscrimination. These include but are not limited to: (a) Title VI of the Civil Rights Act of 1964 (P.L. 88-352) which prohibits discrimination on the basis of race, color or national origin; (b) Title IX of the Education Amendments of 1972, as amended (20 U.S.C. §§ 1681-1683, and 1685-1686), which prohibits discrimination on the basis of sex; (c) Section 504 of the Rehabilitation Act of 1973, as amended (29 U.S.C. § 794), which prohibits discrimination on the basis of handicaps; (d) the Age Discrimination Act of 1975, as amended (42 U.S.C. §§ 6101-6107), which prohibits discrimination on the basis of age; (e) the Drug Abuse Office and Treatment Act of 1972 (P.L. 92-255), as amended, relating to nondiscrimination on the basis of drug abuse; (f) the Comprehensive Alcohol Abuse and Alcoholism Prevention, Treatment and Rehabilitation Act of 1970 (P.L. 91-616), as amended, relating to nondiscrimination on the basis of alcohol abuse or alcoholism; (g) §§ 523 and 527 of the Public Health Service Act of 1912 (42 U.S.C. 290 dd-3 and 290 ee-3), as amended, relating to confidentiality of alcohol and drug abuse patient records; (h) Title VIII of the Civil Rights Act of 1968 (42 U.S.C. § 3601 et seq.), as amended, relating to non-discrimination in the sale, rental or financing of housing; (i) any other nondiscrimination provisions in the specific statute(s) under which application for Federal assistance is being made; and (j) the requirements of any other nondiscrimination statute(s) which may apply to the application.

7. Will comply, or has already complied, with the requirements of Titles II and III of the Uniform Relocation Assistance and Real Property Acquisition Policies Act of 1970 (P.L. 91-646) which provide for fair and equitable treatment of persons displaced or whose property is acquired as a result of Federal or federally assisted programs. These requirements apply to all interests in real property acquired for project purposes regardless of Federal participation in purchases.

8. Will comply with the provisions of the Hatch Act (5 U.S.C. §§ 1501-1508 and 7324-7328) which limit the political activities of employees whose principal employment activities are funded in whole or in part with Federal funds.

9. Will comply, as applicable, with the provisions of the Davis-Bacon Act (40 U.S.C. §§ 276a to 276a7), the Copeland Act (40 U.S.C. § 276c and 18 U.S.C. §§ 874), and the Contract Work Hours and Safety Standards Act (40 U.S.C. §§ 327-33.3), regarding labor standards for federally assisted construction subagreements.

10. Will comply, if applicable, with flood insurance purchase requirements of Section 102(a) of the Flood Disaster Protection Act of 1973 (P.L. 93234) which requires recipients in a special flood hazard area to participate in the program and to purchase flood insurance if the total cost of insurable construction and acquisition is $10,000 or more.

11. Will comply with environmental standards which may be prescribed pursuant to the following: (a) institution of environmental quality control measures under the National Environmental Policy Act of 1969 (P.L. 91-190) and Executive Order (EO) 11514; (b) notification of violating facilities pursuant to EO 11738; (c) protection of wetlands pursuant to EO 11990; (d) evaluation of flood hazards in floodplains in accordance with EO 11988; (e)assurance of project consistency with the approved State management program developed under the Coastal Zone Management Act of 1972 (16 U.S.C. §§ 1451 et seq.); (f) conformity of Federal actions to State (Clear Air) Implementation Plans under Section 176(c) of the Clear Air Act of 1955, as amended (42 U.S.C. § 7401 et seq.); (g) protection of underground sources of drinking water under the Safe Drinking Water Act of 1974, as amended, (P.L. 93-523); and (h) protection of endangered species under the Endangered Species Act of 1973, as amended, (P.L. 93-205).

12. Will comply with the Wild and Scenic Rivers Act of 1968 (16 U.S.C. §§ 1271 et seq.) related to protecting components or potential components of the national wild and scenic rivers system.

13. Will assist the awarding agency in assuring compliance with Section 106 of the National Historic Preservation Act of 1966, as amended (16 U.S.C. 470), EO 11593 (identification and protection of historic properties), and the Archaeological and Historic Preservation Act of 1974 (16 U.S.C. 469a-1 et seq.).

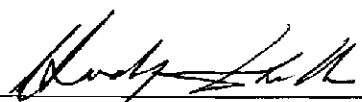
14. Will comply with P.L. 93-348 regarding the protection of human subjects involved in research, development, and related activities supported by this award of assistance.

15. Will comply with the Laboratory Animal Welfare Act of 1966 (P.L. 89-544, as amended, 7 U.S.C. 2131 et seq.) pertaining to the care, handling, and treatment of warm blooded animals held for research, teaching, or other activities supported by this award of assistance.

16. Will comply with the Lead-Based Paint Poisoning Prevention Act (42 U.S.C. §§ 4801 et seq.) which prohibits the use of lead based paint in construction or rehabilitation of residence structures.

17. Will cause to be performed the required financial and compliance audits in accordance with the Single Audit Act of 1995 or OMB Circular No. A-133, Audits of Institutions of Higher Learning and other Non-profit Institutions.

18. Will comply with all applicable requirements of all other Federal laws, executive orders, regulations and policies governing this program.

SIGNATURE OF AUTHORIZED CERTIFYING OFFICIAL	TITLE
	Hardy Dhillon Contract and Grant Officer
APPLICANT ORGANIZATION	DATE SUBMITTED
The Regents of the University of California Box 951406, 1401 Ueberroth Los Angeles, CA 90095-1406	4/15/99

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